Deciphering the association of dysregulated Transcription factors and their cognate microRNAs in search of novel biomarkers for urinary bladder cancer

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LIST OF ABBREVIATIONS

ABBREVIATION	DEFINITION
EMT	Epithelial-Mesenchymal transition
EVs	Extracellular vesicles
TF	Transcription factors
miRNAs	micro Ribonucleic acids
UALCAN	University of ALabama at Birmingham CANcer data analysis portal
TCGA	The Cancer Genome Atlas
GEO	Gene Expression Omnibus
UBC	Urinary Bladder cancer
DEG	Differentially Expressed Genes
TURBT	Transurethral Resection of Bladder Tumour
cfDNA	circulating cell-free DNA
MIBC	Muscle-invasive Bladder Cancer
NMIBC	Non-Muscle-invasive Bladder Cancer
TMT	tri-modality treatment
FGFR	Fibroblast Growth Factor Receptor
ctDNA	Circulating Tumour DNA
STRING	Search Tool for the Retrieval of Interacting Genes/Proteins
DAVID	Database for Annotation, Visualization, and Integrated Discovery
GEPIA	Gene Expression Profiling Interactive Analysis
NGS	Next generation sequencing
qPCR	quantitative polymerase chain reaction
Wnt	wingless int
FC	Fold change
PPI	Protein-protein interaction
MCL	Markov Clustering
MCC	Maximal clique centrality
SOX4	SRY-box transcription factor 4
FOXM1	forkhead box M1

E2F7	**E2F transcription factor 7**
UHRF1	**ubiquitin like with PHD and ring finger domains 1**
E2F2	**E2F transcription factor 2**
E2F8	**E2F transcription factor 8**
E2F1	**E2F transcription factor 1**
MYBL2	**MYB proto-oncogene like 2**
CBX2	**chromobox 2**
SALL4	**spalt like transcription factor 4**
TFAP2A	**transcription factor AP-2 alpha**
ZNF165	**zinc finger protein 165**
HOXB7	**homeobox B7**
IRF5	**interferon regulatory factor 5**
ETV4	**ETS variant transcription factor 4**
WDHD1	**WD repeat and HMG-box DNA binding protein 1**
OTX1	**orthodenticle homeobox 1**
HMGB3	**high mobility group box 3**
HMGB2	**high mobility group box 2**
GRHL2	**grainyhead like transcription factor 2**
NFE2L3	**NFE2 like bZIP transcription factor 3**
TCF21	**transcription factor 21**
EBF1	**Early B Cell Factor 1**
TEAD1	**TEA domain transcription factor 1**
YAP1	**Yes1 associated transcriptional regulator**
HDAC4	**Histone Deacetylase 4**
TCF4	**Transcription Factor 4**
HOXA13	**homeobox A13**
NFIB	**Nuclear Factor I B**
STA5B	**Signal Transducer and Activator of Transcription 5B**
NFATC1	**nuclear factor of activated T cells 1**
PBX3	**Pre-B-Cell Leukemia Homeobox 3**
ATF3	**Activating Transcription Factor 3**

SMARCA2	**SWI/SNF related BAF chromatin remodelling complex subunit ATPase 2**
OSR1	**odd-skipped related transcription factor 1**
ATOH8	**Atonal BHLH Transcription Factor 8**
CBX7	**Chromobox 7**
ZFP2	**zinc finger protein 2**
hsa-miR	**Homo sapines-microRNA**
miRDB	**micro Ribonucleic acids Database**
TNF	**Tumour necrosis factor**
MOB1	**Mps One Binder Kinase Activator-like 1**
TAZ	**Transcriptional coactivator with PDZ-binding motif**
MST1/2	**Mammalian Sterile 20-like Kinase 1 and 2**
WWC	**WW and C2 Domain Containing Proteins**
TGF	**Transforming growth factor**
UED	**Urine exosome Database**
AUC	**Area under curve**

ABSTRACT

Urinary bladder cancer (UBC) continues to be a significant global health issue because of its high rates of incidence, prevalence, and risk of progression to aggressive diseases. Although there have been advancements in diagnostic techniques, existing biomarkers do not provide adequate sensitivity and specificity for early detection and prognostic assessment. Transcription factors (TFs) and microRNAs (miRNAs) play crucial roles in regulating gene expression and often operate within intricate regulatory networks. High-throughput transcriptomic datasets from The Cancer Genome Atlas (TCGA), Gepia2.0 and Gene Expression Omnibus (GEO) were mined to identify differentially expressed TFs and miRNAs. Target prediction databases including UALCAN, miRDataBase, and STRING were employed to map functional TF-miRNA regulatory pairs. The disruption of TF-miRNA interactions has been linked to various cancers, but their coordinated contributions to the development of UBC are still not fully understood. This research aims to elucidate the relationship between abnormal levels of TFs and their corresponding miRNAs to pinpoint reliable molecular markers for BLCA. Comprehensive bioinformatics analyses were conducted using publicly available transcriptomic datasets to identify TFs and miRNAs that are differentially expressed. Network construction using miRTargetLink2.0 highlighted critical regulatory nodes with high centrality scores. Target prediction algorithms, correlation analyses, and network topology assessments were utilized to build and examine TF-miRNA regulatory networks. Functional enrichment and pathway analyses demonstrated the participation of identified TF-miRNA pairs in oncogenic mechanisms such as epithelial-mesenchymal transition (EMT), cell growth, apoptosis, and immune regulation. Key regulatory nodes were selected based on the network centrality and verified using independent clinical datasets. Furthermore, survival analysis using Kaplan-Meier/log rank test demonstrated the potential of particular TF-miRNA patterns as prognostic and diagnostic biomarkers by linking them to patient outcomes. The present study establishes a foundation for future functional investigations and therapeutic targeting of essential TF-miRNA regulatory network in urinary bladder cancer.

Keywords: Bioinformatics; Transcription factors (TFs); miRNA; bladder cancer; diagnostic biomarkers; differential gene expression.

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1 Introduction

Urinary bladder cancer (UBC) stands as a prevalent malignancy globally, characterized by high recurrence rates and significant molecular heterogeneity. Despite advancements in therapeutic strategies, the prognosis for UBC patients remains suboptimal, largely due to the lack of reliable molecular biomarkers for early detection and prognosis. In recent times researchers have emphasized the essential functions of transcription factors (TFs) and microRNAs (miRNAs) within the complex regulatory systems that control gene expression, with their improper regulation playing a role in the development of tumours and the advancement of cancer.

Transcription factors (TFs) are pivotal in modulating gene expression by binding to specific DNA sequences, thereby influencing various cellular processes. In the context of UBC, aberrant expression of TFs such as YAP, TEAD1, NFIB and RB1 haves been implicated in tumour development and progression (included the upregulated TFs as well. Conversely E2F1, part of the E2F family, is often found to be overexpressed in UBC, facilitating cell cycle advancement and tumour growth. Likewise, SOX4 has been recognized as an oncogenic transcription factor in BC, playing a role in epithelial-mesenchymal transition and metastasis. Concurrently, miRNAs, small non-coding RNAs, regulate gene expression post-transcriptionally and have been identified as either oncogenes or tumour suppressors in various cancers, including UBC. The interplay between TFs and miRNAs forms complex regulatory circuits that can amplify or suppress oncogenic signals, thereby influencing cancer cell behaviour.

Recent advances in cancer biology have increasingly emphasized the pivotal role of genetic alterations and dysregulated gene expression in the initiation, progression, and heterogeneity of urinary bladder cancer. The integration of high-throughput omics technologies—such as RNA sequencing and microarray profiling—with robust bioinformatics pipelines has enabled the identification of differentially expressed genes (DEGs) that are integral to the molecular pathogenesis of bladder cancer. These molecular insights have revolutionized diagnostic and therapeutic strategies, particularly through the identification of novel biomarkers with potential utility in early detection, prognosis, and targeted therapy.

Despite these molecular advancements, the clinical management of bladder cancer continues to rely heavily on traditional diagnostic modalities. Transurethral resection of bladder tumour

(TURBT) and cystoscopy remain the gold standards for the initial diagnosis and surveillance of bladder cancer. However, these methods are invasive, costly, and associated with procedural discomfort and risks of complications. Tissue biopsy, while essential for histopathological confirmation and tumour grading, presents limitations including sampling bias, inability to capture tumour heterogeneity, and challenges in repeated sampling during disease monitoring.

These constraints highlight the pressing need for non-invasive, yet accurate, diagnostic and prognostic alternatives. In this context, liquid biopsy—based on the analysis of circulating biomarkers such as cell-free DNA (cfDNA), circulating tumour cells (CTCs), and extracellular vesicle (EVs) including exosomes—has emerged as a promising tool. Liquid biopsy offers a minimally invasive approach for real-time monitoring of tumour dynamics, enabling the detection of genetic and epigenetic alterations associated with disease progression, recurrence, and therapeutic resistance. As a result, ongoing research increasingly focuses on the identification and validation of molecular signatures, including transcription factors and their regulatory microRNAs (miRNAs) that can be effectively detected through liquid biopsy to enhance the early diagnosis and personalized management of bladder cancer.

Recent bioinformatics analyses have shed light on the TF-miRNA regulatory networks in UBC. In recent study by Wang *et a*l. (2022) identified a miRNA-mRNA regulatory pair, miR-17-5p and TGFBR2, which plays a significant role in UBC pathogenesis and holds potential for clinical applications in diagnosis and tumour immunity. Furthermore, network analyses have revealed that TFs like PTEN are targeted by multiple miRNAs, including hsa-miR-19a, forming self-regulatory loops that are differentially expressed in bladder cancer, suggesting their potential as biomarkers.

The stipulated interaction between miRNAs and signalling pathways, including the Hippo circuit, Wnt/β-catenin, and STAT3, underscores the diverse functions of miRNAs in regulating pathways linked to cancer. Deregulation of these pathways contributes to epithelial-mesenchymal transition (EMT), proliferation, and apoptosis evasion in UBC cells.

Given the complexity and significance of TF-miRNA interactions in UBC, this study aims to systematically decipher these associations using integrated bioinformatics approaches. By identifying differentially expressed TFs and their cognate miRNAs, constructing regulatory networks, and performing functional enrichment analyses, we seek to uncover novel molecular biomarkers that can enhance the diagnostic and prognostic landscape of urinary bladder cancer.

2 Review of Literature

2.1. Overview of Urinary Bladder Cancer

Urinary bladder cancer (UBC) is among the most prevalent cancers worldwide, exhibiting a notable rate of recurrence and progression in numerous patients. It stands as the 10th most frequently diagnosed cancer with an estimated 573,000 new cases and over 200,000 fatalities each year (Sung et al., 2021). It is known for its high rates of recurrence and advancement, particularly in cases of non-muscle invasive bladder cancer (NMIBC), which represents about 75% of initial diagnoses. Although muscle-invasive bladder cancer (MIBC) is less frequently diagnosed at first, it has significantly worse prognosis and typically necessitates intense treatment options like radical cystectomy, systemic chemotherapy and increasingly bladder-sparing approaches using Trimodal therapy (TMT), which combines transurethral resection, radiotherapy, and concurrent chemotherapy. Treatment approaches have progressed beyond traditional chemotherapy and surgical techniques. The incorporation of immunotherapy, particularly immune checkpoint inhibitors (such as Atezolizumab and Pembrolizumab), has markedly enhanced survival rates in patients dealing with advanced or metastatic bladder cancer. Moreover, antibody-drug conjugates (like Enfortumab Vedotin) and FGFR inhibitors (such as Erdafitinib) have shown effectiveness in Patients selected based on molecular characteristics, particularly those with specific genetic changes like FGFR3 mutations. Recent genomic investigations have uncovered considerable molecular diversity in urothelial bladder cancer (UBC). Transcriptomic assessments have also categorized bladder cancers into various molecular subtypes, primarily luminal and basal/squamous, each exhibiting unique therapeutic vulnerabilities and prognostic outcomes. Despite progress in treatment modalities, including surgery, chemotherapy, immunotherapy, and targeted therapies, high rates of recurrence and resistance to treatment persist as major obstacles. Consequently, ongoing research is increasingly focused on developing non-invasive diagnostic techniques such as liquid biopsy, which examines circulating tumour DNA (ctDNA), exosomes, and cell-free miRNAs.

2.2. Molecular Biomarkers

Molecular biomarkers have become vital instruments for enhancing the diagnosis, prognosis, and monitoring of urinary bladder cancer (UBC). Altered transcription factors (TFs) and their corresponding microRNAs (miRNAs) are increasingly acknowledged as important molecular indicators. Research has uncovered several decreased TFs, including TCF21, EBF1, TEAD1, and HOXA13, along with increased TFs like SOX4, FOXM1, E2F7, and SALL4 in UBC, indicating their involvement in oncogenic transformation and tumour advancement. These TFs

affect various cellular pathways, such as cell cycle control, epithelial-mesenchymal transition, and immune evasion.

MiRNAs modulated by these TFs also play a role in tumour behaviour, making TF-miRNA interactions promising candidates for biomarkers. Recent studies emphasize the potential of liquid biopsy techniques, which involve detecting circulating miRNAs and transcriptional signatures in body fluids, providing non-invasive, real-time information on tumour behaviour. Utilizing bioinformatics resources like STRING and miRDB facilitates the discovery of clinically significant TF-miRNA relationships. Overall, molecular biomarkers centered within these dysregulated TFs and miRNAs represent a substantial opportunity for improving early detection and personalized treatment of bladder cancer.

2.3. Transcription Factors in Cancer Development

Transcription factors (TFs) are master regulators of gene expression that oversee essential cellular functions like proliferation, differentiation, apoptosis, and migration. The dysregulation of TFs—whether through increased or decreased expression—has been associated with cancer development, tumour growth, metastasis, and resistance to treatment. Various studies have pointed out the significance of TFs in the biology of UBC.

Recent research has pinpointed TFs such as FOXM1, SOX4, and certain members of the E2F family as significant contributors to various cancers, including urinary bladder cancer, where they facilitate unchecked cell cycle advancement and invasion.

Changes in TF activity can result from genetic alterations, epigenetic changes, or disrupted signalling pathways, leading to extensive transcriptional reprogramming in cancerous cells. Additionally, TFs frequently interact with non-coding RNAs, particularly microRNAs, forming intricate regulatory networks that amplify tumour aggressiveness and resistance to treatment. Progress in transcriptomic analysis and bioinformatics tools has allowed to identify specific TFs as prospective biomarkers and therapeutic targets. Therefore, comprehending TF dysregulation provides critical insights necessary for developing early diagnostic methods and targeted treatments in the management of cancer.

2.3.1 Upregulated Transcription Factors in UBC

• SOX4, FOXM1, E2F7, UHRF1, E2F2, E2F8, E2F1, MYBL2, CBX2, SALL4, and TFAP2A. These TFs are often linked with pathways concerning cell growth, chromatin restructuring, and DNA repair, which are frequently manipulated during the progression of cancer.

2.3.2 Downregulated Transcription Factors in UBC

12 downregulated TFs were selected from a pool of over 2500 dysregulated genes. These are:

• TCF21, EBF1, TEAD1, YAP1, HDAC4, TCF4, HOXA13, NFIB, STAT5B, NFATC1, PBX3, and ATF3. These TFs typically play significant roles in differentiation, cell cycle regulation, and apoptosis. Their reduced expression may lead to the weakening of tumour-suppressive capabilities.

2.4 The Function of microRNAs in Regulating TFs

MicroRNAs (miRNAs) are short non-coding RNAs that are essential for the post-transcriptional regulation of gene expression. In context of cancer, miRNAs have a significant effect on transcription factors (TFs) by either directly degrading their mRNAs or inhibiting their translation. This regulation by miRNAs results in intricate feedback and feed-forward loops that precisely adjust TF activity, influencing crucial cancer-related processes such as cell proliferation, epithelial-mesenchymal transition, angiogenesis, and evasion of the immune system. Imbalances in these miRNAs can result in persistent activation of transcription factors, which may drive cancer advancement and resistance to treatment. The development of bioinformatics tools like miRDB has enabled the mapping of miRNA-transcription factor interaction networks, providing valuable understanding of tumour biology. Focusing on disrupted miRNA-transcription factor interactions presents a great opportunity for creating new biomarkers and targeted treatments, especially through non-invasive liquid biopsy methods for the early detection and ongoing assessment of cancer.

2.5 Bioinformatics Tools in Discovering Markers

This research employed several bioinformatics tools:

•**GEPIA2.0** (Gene Expression Profiling Interactive Analysis) **http://gepia.cancer-pku.cn/** is a web-based tool that offers interactive analysis of RNA sequencing data derived from TCGA and GTEx projects. It enables users to conduct gene expression profiling, survival analyses, and correlation studies involving cancerous and normal samples. GEPIA proves to be especially useful for cancer research, enabling quick confirmation of gene expression patterns across various cancer types.

•**DAVID** at **https://david.ncifcrf.gov/** (Database for Annotation, Visualization, and Integrated Discovery) is a bioinformatics resource that offers functional annotation and enrichment analysis for extensive gene sets. It assists in pinpointing important transcription factors and regulatory components by examining gene lists. This tool aids in identifying essential

regulatory networks and comprehending the transcriptional regulation associated with conditions like cancer.

•**Cytoscape3.10.3 (https://cytoscape.org/)** is an open-source software program used to visualize and examine intricate networks of molecular interactions. It makes possible to discover important regulatory nodes, like hub transcription factors (TFs), by integrating data on gene expression and protein-protein interactions. Tools like CytoHubba and MCODE in Cytoscape aid in detection of highly linked genes in cancer research, providing information on disease causes and possible biomarkers. Cytoscape was utilized in this study to examine HubTF–miRNA networks and identify key TFs linked to the advancement of bladder cancer. Because of its adaptability, it plays a crucial role in network-based biomarker discovery and systems biology.

• The **STRING** (Search Tool for the Retrieval of Interacting Genes/Proteins) (**https://string-db.org/)** database was used to explore TF interactions and their relevance to UBC. It helps in identifying functional associations between proteins, including transcription factors, for understanding molecular mechanisms pathways interrelated and how they are strongly connected to each other.

• **miRDB** (http://mirdb.org/**)** was utilized to forecast miRNA–TF relationships It is a web-based bioinformatics platform that forecasts interactions between microRNAs and their target genes. It enables researchers to investigate regulatory networks of miRNAs and pinpoint specific miRNAs linked to particular genes, such as transcription factors. miRDB offers important information useful for discovering biomarkers and identifying potential therapeutic targets.

•**miRTargetLink2.0** (https://www.mirtargetlink.org/**)** is a bioinformatics resource that delivers extensive information regarding interactions between microRNAs and their target transcription factors (TFs). It combines data from multiple sources, providing insights into how miRNAs regulate gene expression. This tool is essential for investigating miRNA-influenced gene networks in context of diseases.

2.6 Liquid Biopsy as a Diagnostic Modality

Liquid biopsy involves the analysis of body fluids, such as blood or urine, to detect cancer biomarkers. This technique has surfaced as a ground breaking, minimally invasive diagnostic method, allowing for the real-time observation of tumour by examining circulating biomarkers such as cell-free DNA (cfDNA), circulating tumour DNA (ctDNA), exosomal RNA, and miRNAs. For urinary bladder cancer (UBC), liquid biopsy presents a valuable substitute for

conventional invasive techniques such as cystoscopy and tissue biopsy, making early detection, prognosis evaluation, and treatment monitoring more accessible.

Recent research has shown the effectiveness of urine-based liquid biopsy in detecting molecular markers in patients with muscle-invasive bladder cancer (MIBC), underlining its potential utility in clinical practice. Furthermore, developments in next-generation sequencing (NGS) and bioinformatics have improved the accuracy and reliability of identifying tumour-specific modifications, including altered transcription factors (TFs) and their related miRNAs, through liquid biopsy.

Incorporating liquid biopsy into clinical practice has the potential to transform the management of bladder cancer by facilitating tailored treatment approaches, minimizing the necessity for invasive procedures, and providing ongoing disease surveillance.

2.7 Research Gap and Original Contribution of the Study

While numerous studies have looked at TFs or miRNAs separately in UBC, few have integrated the TF–miRNA interaction and assessed its potential for early diagnostics through liquid biopsy. This research addresses this gap by:

• Systematically pinpointing dysregulated TFs in UBC.

• Mapping their corresponding miRNAs using in-silico analysis and bioinformatics tools.

• Suggesting a non-invasive method for detection using liquid biopsy.

2.8 Future Directions

• Experimental verification of the TF–miRNA signatures in liquid biopsy samples obtained from patients.

• Creation of multiplex assays (such as qPCR panels) for concurrent detection.

• Assessment of sensitivity, specificity, and clinical significance of these markers in larger cohorts.

3 Hypothesis

An integrated bioinformatics analysis of dysregulated transcription factors and their cognate miRNAs and construction of a functionally relevant regulatory networks in urinary bladder cancer can reveal novel, non-invasive biomarkers.

4 Key Questions

- Which transcription factors are significantly dysregulated in urinary bladder cancer according to transcriptomic datasets and literature evidence?
- What are the specific miRNAs associated with these dysregulated TFs based on validated prediction databases?
- How do these TF–miRNA interactions contribute to oncogenic mechanisms like EMT, apoptosis, or immune evasion in bladder cancer?
- Can the TF–miRNA networks be mapped to identify key regulatory nodes with biomarker potential?
- Is it feasible to detect these TF–miRNA signatures non-invasively via liquid biopsy in clinical settings?

5 Objectives

- **Data Mining and Filtering:**
    1. To extract transcription factor genes dysregulated in bladder cancer using RNA-seq and microarray datasets
    2. To shortlist significantly deregulated TFs through literature curation and STRING-based interaction analysis.
- **miRNA Association Analysis:**
    1. To identify miRNAs predicted or validated to target the selected TFs using databases like miRDB and miRTarBase.
- **Network Construction and Functional Analysis:**
    1. To build TF–miRNA regulatory networks using miRTargetLink.
    2. To identify hub nodes and assess their network centrality and role in known cancer pathways (e.g., Hippo, Wnt/β-catenin, STAT3).
- **Biomarker Discovery :**
    1. To evaluate the potential of identified TF–miRNA pairs as minimally invasive biomarkers by assessing their detectability in biofluids (urine, blood).

6 Chapter 1

6.1 Objective: Data Mining and Filtering of Dysregulated Transcription Factors in Bladder Cancer

6.2 Materials and Methods

6.2.1 The initial Sorting and Dataset Recovery

High-confidence databases like TCGA (The Cancer Genome Atlas) and GEO (Gene Expression Omnibus) were explored for publicly accessible RNA-seq and microarray gene expression datasets relevant to bladder cancer. Datasets that profiled transcriptional alterations between bladder cancer samples and adjacent normal tissues were the main priority.

- RNA-seq data: We primarily focused on The Cancer Genome Atlas (TCGA)-BLCA data.
- For further information, GEO microarray datasets including GSE13507, GSE7476, and GSE31684 were examined.

Using predetermined cut-offs, substantially dysregulated genes were found in these datasets:

- **Fold Change (FC):** >2 or <0.5
- **p-value (adjusted):** <0.05

A list of more than 2500 genes with differential expression was the result.

6.2.2 Identification of DNA Binding Factors and annotating the Transcription Factors

Using the DAVID bioinformatics tool database, the list of dysregulated genes was compared with a curated human transcription factor database. Following this filtering procedure, a list of DNA binding factors, dysregulated in bladder cancer was generated.

A total of 225 DNA Binding Factors were shortlisted from those dysregulated genes by using the DAVID bioinformatics tool. Out of those 225, 85 were upregulated and 140 were downregulated DNA Binding factors. Next, we annotated those DNA Binding factors as Transcription factors. Finally, 96 downregulated TFs and 38 upregulated TFs were categorized.

6.2.3 Literature Curation

Using PubMed, Scopus, and Google Scholar, a manual literature curation process was carried out to confirm the discovered TFs' role in the pathophysiology of bladder cancer. The inclusion criteria were:

- Experimentally confirmed bladder cancer and its dysregulated TFs.
- Relationship with the stage, prognosis, tumour grade, or resistance to treatment.

With the use of this stage of research, the TFs were narrowed down to **21** significant transcription factors that were upregulated and **17** that were downregulated, all of which had reliable literature reports.

6.2.4 STRING Database Interaction Analysis

The STRING v11.5 database was used to create protein–protein interaction (PPI) networks for both upregulated and downregulated TFs. MCL clustering techniques were used to identify functionally enriched clusters and depict high-confidence interactions (score >0.7).

6.3 Results

6.3.1 Summary of TF Mining

Table 1: List of TFs shorted from Gepia2.0 to DAVID till Literature curation

Source	Number of Differentially Expressed Genes	Dysregulated DNA binding factor (DAVID)	Dysregulated TFs Identified	Shortlisted TF (By literature curation)
TCGA,GEO and GEPIA	~2500	225 (85 upregulated and 140 downregulated)	134 (38 upregulated and 96 downregulated)	38 (21 upregulated and 17 downregulated)

6.3.2 Shortlisted Transcription Factors

Upregulated TFs:

- SOX4, FOXM1, E2F7, UHRF1, E2F2, E2F8, E2F1, MYBL2, CBX2, SALL4, TFAP2A, ZNF165, HOXB7, IRF5, ETV4, WDHD1, OTX1, HMGB3, HMGB2, GRHL2, NFE2L3.

Table 2: Differential Expression Metrics of Significantly Upregulated TFs in Bladder Cancer

TF(ID)	Median(Tumour)	Log2Fold change	P value
SOX4	31.775	1.849	1.21E-11
FOXM1	29.565	3.555	2.38E-20
E2F7	1.545	1.014	4.41E-06
UHRF1	14.465	2.925	1.96E-24
E2F2	1.92	1.254	5.72E-06
E2F8	4.275	1.485	1.02E-06
E2F1	12.22	2.176	1.90E-10
MYBL2	36.015	4.086	1.52E-23
CBX2	4.305	1.459	1.63E-07
SALL4	1.81	1.151	6.98E-07
TFAP2A	15.264	1.726	2.29E-04
ZNF165	4.17	1.187	2.58E-05
HOXB7	26.195	1.398	1.13E-09
IRF5	16.44	1.213	4.21E-06
ETV4	16.26	2.402	1.73E-10
WDHD1	6.145	1.541	6.05E-11
OTX1	1.705	1.386	1.76E-11
HMGB3	80.643	1.973	2.48E-08
HMGB2	147.027	1.001	5.30E-04
GRHL2	17.43	1.105	4.42E-08
NFE2L3	14.46	1.28	1.05E-06

Downregulated TFs:

- TCF21, EBF1, TEAD1, YAP1, HDAC4, TCF4, HOXA13, NFIB, STAT5B, NFATC1, PBX3, ATF3, ZFP2, SMARCA2, OSR1, ATOH8, CBX7.

Table 3: Differential Expression Metrics of Significantly Downregulated TFs in Bladder Cancer

TF(ID)	Median(Tumour)	Log2 Fold Change	P value
TCF21	0.725	-3.013	4.31E-44
EBF1	0.69	-262	1.69E-27
TEAD1	6.605	-1.905	1.05E-13
YAP1	24.035	-1.287	2.89E-05
HDAC4	24.035	-1.261	1.41E-19
TCF4	3.98	-1.768	5.89E-09
HOXA13	2.465	-1.757	1.14E-04
NFIB	4.095	-1.667	9.24E-10
STAT5B	13.9	-1.557	8.32E-16
NFATC1	3.125	-1.150	1.47E-07
PBX3	8.395	-1.144	1.11E-06
ATF3	31.194	-2.098	2.01E-10
SMARCA2	27.045	-1.046	1.66E-05
OSR1	6.125	-2.659	1.25E-06
ATOH8	2.275	-2.847	4.02E-06
CBX7	6.47	-2.802	3.59E-29
ZFP2	0.28	-1.165	1.30E-22

6.3.3 Analysis of STRING Networks

Upregulated TF Network:

Upregulated TFs created a close-knit interaction network centered around the E2F family and FOXM1, establishing a core regulatory module in cell proliferation, DNA repair, and mitotic progression in bladder cancer.

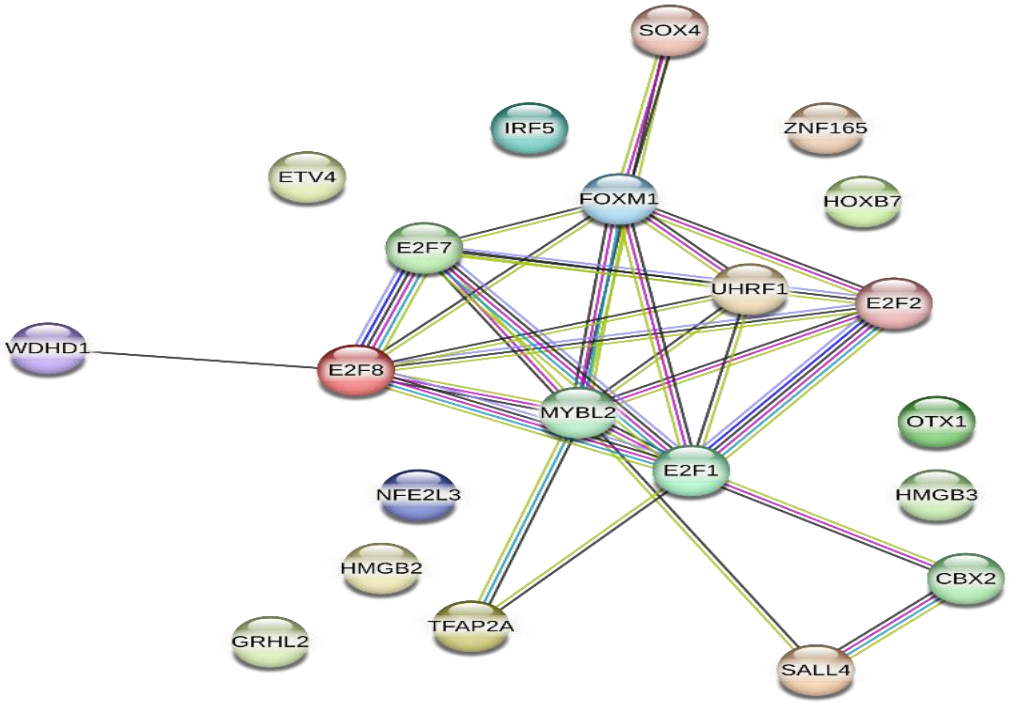


Fig 1: Upregulated TFs in STRING Network analysis

Downregulated TF Network:

Several TFs, including TCF21 and HOXA13, were reported to have high co-expression and experimental interaction evidence, according to the STRING interaction map. The network revealed a significant cluster associated with differentiation and epithelial-to-mesenchymal transition (EMT) control.

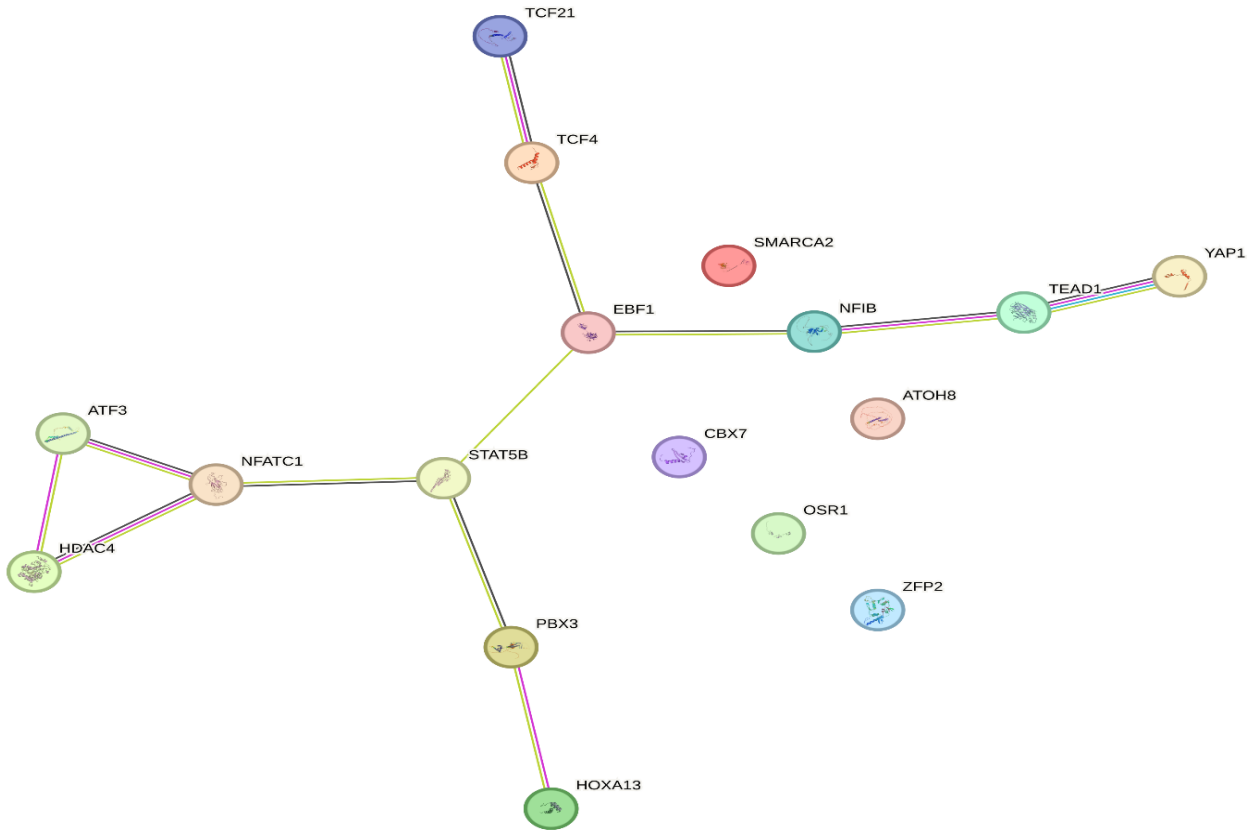


Fig 2: Downregulated TFs in STRING Network analysis

6.4 Discussion

The data mining technique effectively extracted a physically relevant collection of dysregulated transcription factors in bladder cancer from a large expression dataset.

Key regulatory nodes with possible diagnostic and therapeutic implications were represented by the last 38 TFs on the list.

- **TCF21** and **HOXA13** are examples of **downregulated TFs** that have been linked in the past to tumour suppression, differentiation control, and the suppression of metastatic characteristics. The loss of differentiation signals in cancer is suggested by their persistent downregulation.
- **Upregulated TFs**, especially those from the **FOXM1** and **E2F** families, constitute a mitogenic module that may be causing bladder cancer cells to proliferate uncontrollably. These might serve as the targets of therapeutic action.

The functional association between these TFs has been determined by **STRING** analysis, enhancing their potential for further molecular research and exploring novel biomarkers.

6.5 Conclusion

This work effectively discovered a targeted panel of transcription factors (TFs) that were markedly deregulated in bladder cancer by means of rigorous literature curation and integrated analysis of high-throughput transcriptome datasets. Based on their known functions in tumour biology and expression patterns, a total of 23 TFs—11 upregulated and 12 downregulated candidates—were shortlisted. Using the STRING database, protein-protein interaction analysis showed strong functional relationships, especially between TFs linked to differentiation and proliferation. The biological significance of these transcription factors in the pathological process of bladder cancer was highlighted by these findings, which also establishes a framework for the study's subsequent phase, to map their regulatory connections with relevant microRNAs. The development of non-invasive diagnostic and prognostic procedures may benefit greatly from the use of this selected array of TFs as a molecular signature.

7 Chapter 2

7.1 Objective: The study of miRNA associations with the dysregulated transcription factors with Establishing Networks and Analyzing TF–miRNA Regulatory Interactions Functionally in bladder cancer

7.2 Materials and Methods

7.2.1miRNA Target Prediction

Two highly reliable miRNA–target prediction databases were utilized to examine the post-transcriptional regulation of the transcription factors (TFs) that were shortlisted:

- **miRDB** (http://mirdb.org/): For each TF, miRNAs having a target score of ≥80 were predicted.
- **miRTarBase**: Verified miRNA–TF interactions using experimental data (CLIP-seq, Western blotting, reporter assays).

The above databases were accessed for each of the 23 dysregulated TFs (11 upregulated, 12 downregulated) following STRING analysis. Only the high-confidence miRNA interactions that had strong prediction scores or proven experimental support were taken into consideration.

7.2.2 Constructing and Visualizing Networks

Using the **miRTargetLink2.0** platform (https://www.mirtargetlink.org/), a comprehensive interaction network was generated for:

- Upregulated TFs and their negatively regulating miRNAs.
- Downregulated TFs and their potentially elevated miRNAs

Cytoscape3.10.3 was used to visualize networks, highlighting nodes of biological significance in bladder cancer and key miRNAs that have multiple TF targets (hubs).

Network elements:

- **Nodes**: miRNAs, TFs, and genes that have been validated experimentally.
- **Edges**: Indicate inhibitory/activating regulatory links.

7.2.3 Identification of Hub Transcription Factors Using CytoHubba in Cytoscape

Using Cytoscapev3.10.3 and the CytoHubba plugin, functionally significant transcription factors (TFs) were ranked in the protein–protein interaction (PPI) networks constructed from

the STRING database. Maximal Clique Centrality (MCC), a topological metric that is known to successfully identify important proteins in biological networks, was used (Chin et al., 2014).

- First, a.tsv file containing the STRING PPI network (confidence score >0.7) for both upregulated and downregulated TFs was exported.
- Networks were imported into Cytoscape and processed through **CytoHubba**.
- Based on MCC scores, the top three hub transcription factors (upregulated/downregulated) were chosen for each group.
- Nodes were color-coded according to importance, with red indicating the **top-ranked hub TF**.

7.2.4 Network Topology and Hub Node Detection

Network characteristics were calculated via Network Analyzer in **miRTargetLink2.0**;

- **Degree centrality**: It is the direct connections that a node has.
- **Betweenness centrality**: Influence over flow of information in the network.
- **Closeness centrality**: Speed of information within a node.

Hub nodes have been determined as top 10% of centrality value.

7.2.5 Functional Annotation and Filtering

- According to their deregulation state in bladder cancer, miRNAs were further filtered from literature and sorted as unique and duplicate miRNAs (e.g., Wang et al., 2022, Zhang et al., 2021).
- DIANA-**mirPath** and **miRNet** for central nodes were used to identify functional pathways (such as EMT, cell cycle, and immune evasion).

7.3 Results

7.3.1 TF Network and Hub gene detection by cytoscape3.10.3 through cytohubba

- **Downregulated TF Network and Hub Detection**

At the top hub, EBF1 was linked to six other TFs (including TCF4, NFIB, and PBX3), indicating that it plays a crucial role in the differentiation and transcriptional repression processes in bladder cancer. T-cell activation and cytokine signalling are known to be regulated by the second and third hubs, respectively, STAT5B and NFATC1. Because of their central location, bladder cancer may be caused by an aberration in the immune-modulatory axis.

Table 4: Downregulated TFs with its Rank and MCC score

Rank	Transcription Factor	MCC Score
1	EBF1	5
2	STAT5B	4
3	NFATC1	3

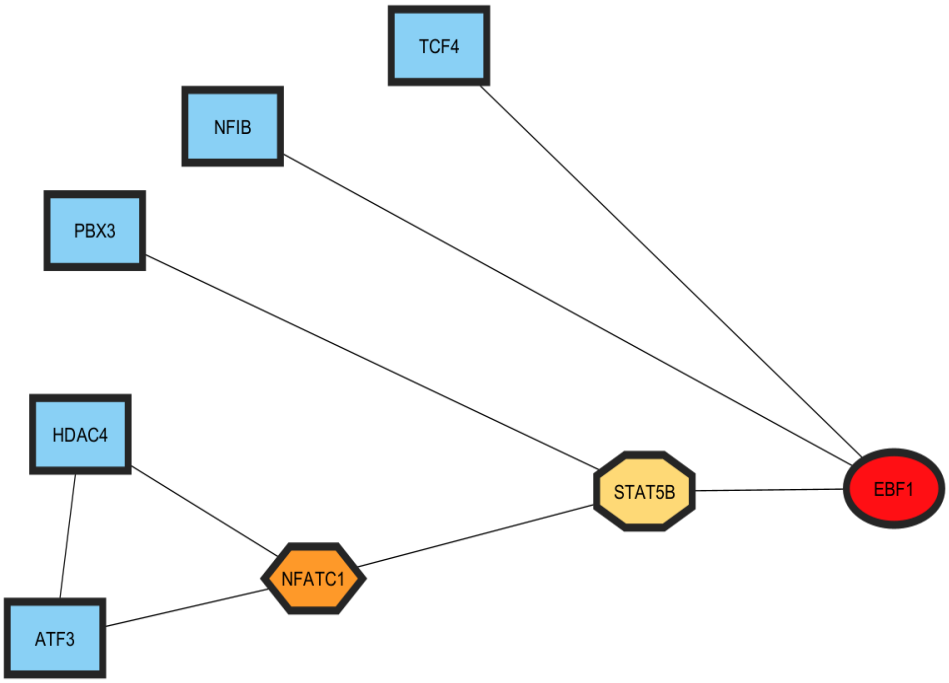


Fig 3: CytoHubba-based Hub Detection in Downregulated TF Network

- **Upregulated TF Network and Hub Detection**

The main hub transcription factor was found to be MYBL2, which is closely related to FOXM1, chromatin modifiers such as CBX2 and UHRF1, and members of the E2F family (E2F1, E2F2, E2F7, and E2F8). In line with earlier findings in the literature on bladder cancer, its centrality emphasizes its crucial role in chromatin remodelling, cell cycle regulation, and tumour growth (Singh et al., 2022; Wang et al., 2021).

The well-known oncogenic TF E2F1 demonstrated direct connections with almost all other upregulated TFs, confirming its function in DNA replication and the G1/S phase transition. A profound connection was also shown by FOXM1, a master regulator of mitosis, which is in line with its function in high-grade bladder cancers.

Table 5: Upregulated TFs with its Rank and MCC score

Rank	Transcription Factor	MCC Score
1	MYBL2	10
2	E2F1	9
3	FOXM1	7

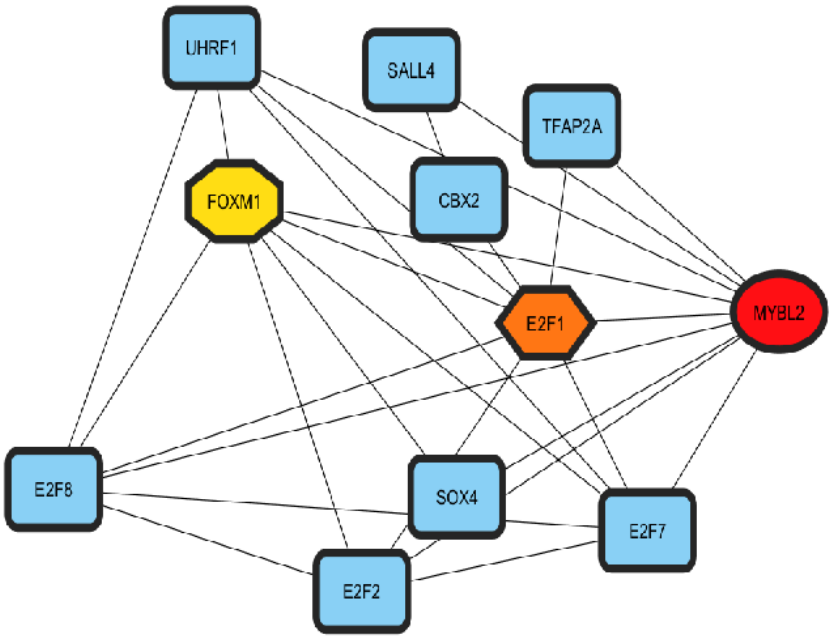


Fig 4: CytoHubba-based Hub Detection in Upregulated TF Network

7.3.2 Interpretation and Functional Implications of Hub TFs

These TFs' topological dominance in the interaction networks, which is corroborated by their biological significance, indicates that they are not only functionally relevant nodes but could also be potential targets for biomarker emergence and therapeutic intervention.

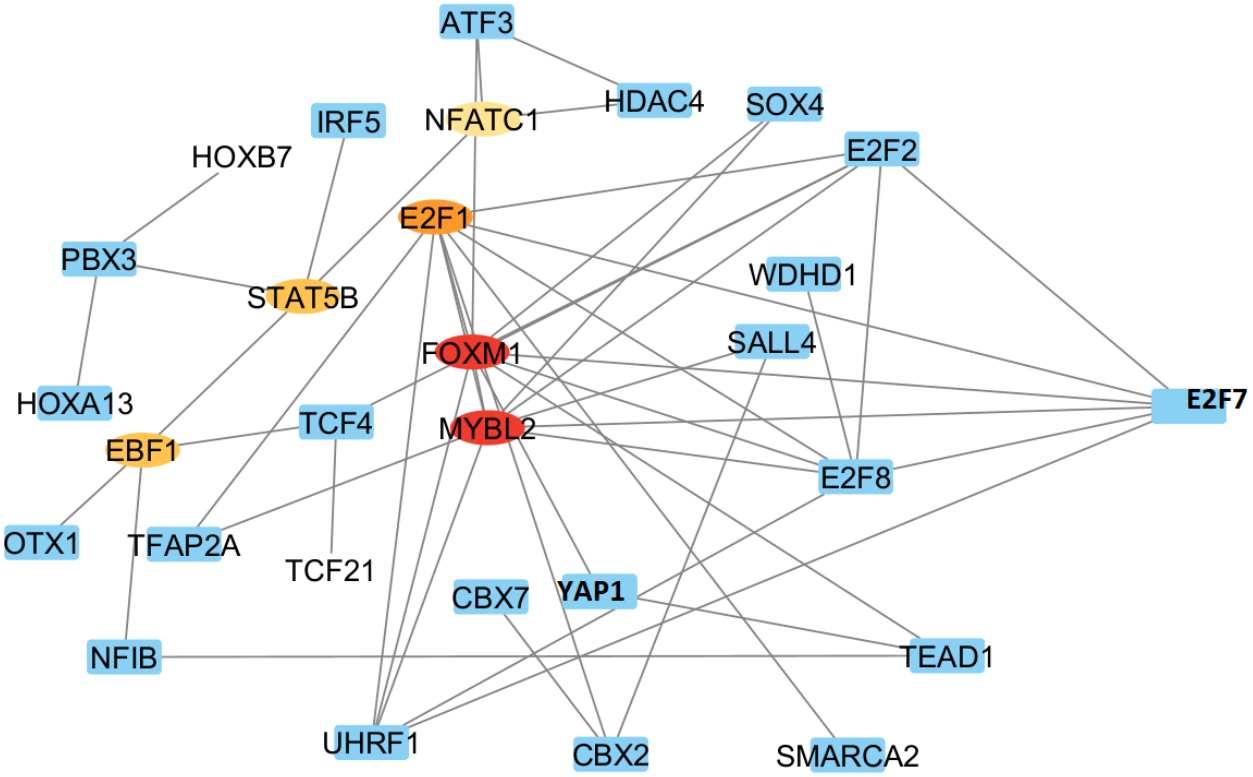


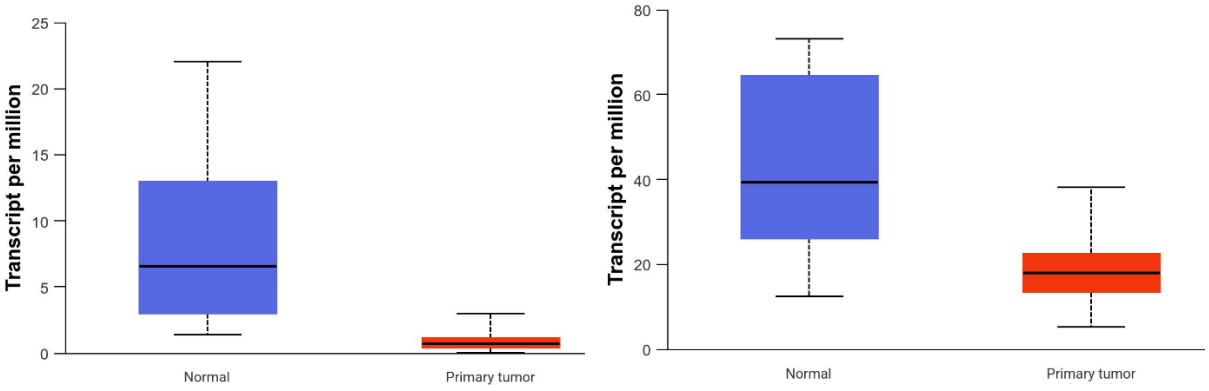
Fig 5: The top hub genes were selected based on degree centrality using the CytoHubba tool in Cytoscape.

Table 6: Functional classification of key hub TFs

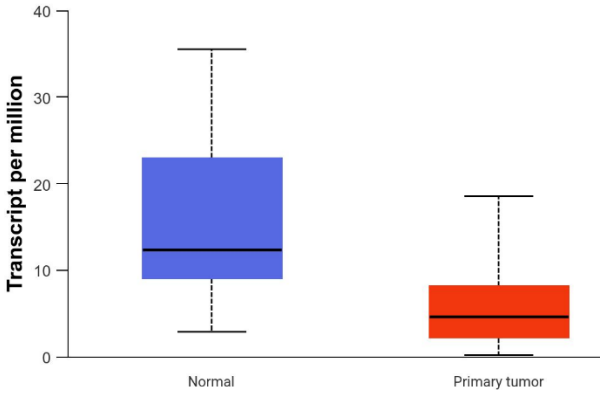
TF Category	**Top Hub TFs**	**Biological Roles**	**Network Involvement**
Downregulated	EBF1, STAT5B, NFATC1	Differentiation, immune signalling, tumour suppression	Disruption may promote de-differentiation and immune evasion
Upregulated	MYBL2, E2F1, FOXM1	Cell cycle progression, chromatin dynamics, proliferation	Central to tumour growth and aggressiveness

7.3.3 Expression Validation of Hub Transcription Factors in TCGA-BLCA Dataset

- **Expression Validation OF Downregulated TFs**



(A) **(B)**



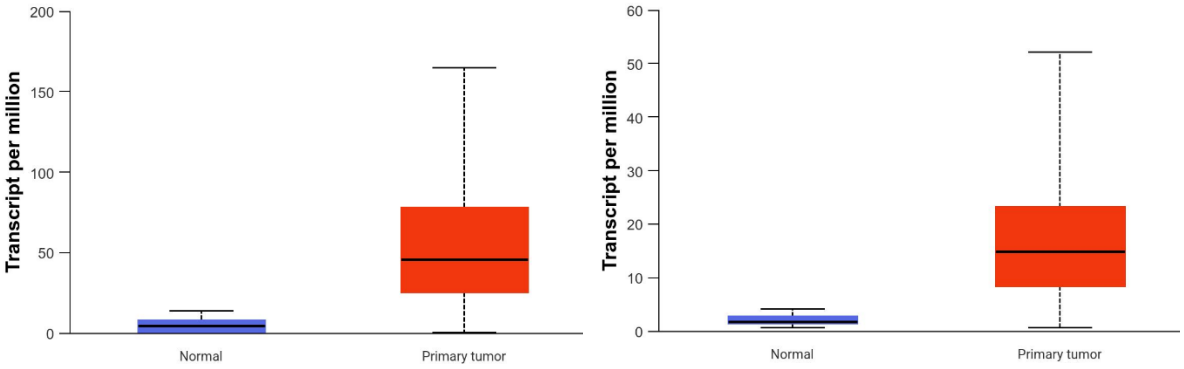
(C)

Fig 6: Box plot depicting the downregulated TFS expression levels in normal vs primary tumour samples in Bladder Urothelial Carcinoma (TCGA-BLCA) (A)-EBF1 (B)-STAT5B (C)-NFATC1

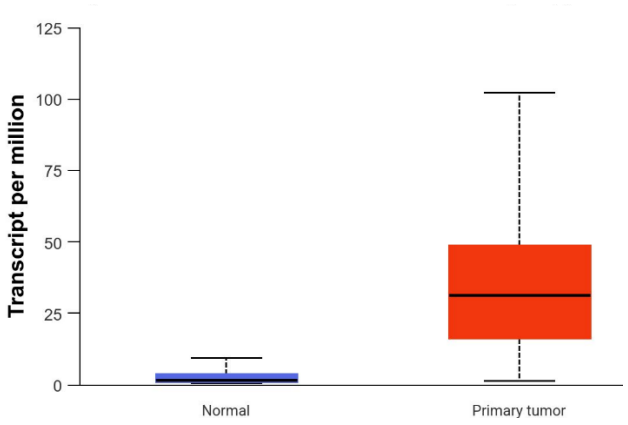
Table 7: Median Expression comparison of significantly downregulated (Hub) TFs between Normal vs tumour samples

TF	Regulation	Median(Normal)	Median(Tumour)
EBF1	down	6.575	0.692
STAT5B	down	39.342	17.194
NFATC1	down	12.295	4.650

- **Expression Validation OF Upregulated TFs**



(A) **(B)**



(C)

Fig 7: Box plot depicting the upregulated TFS expression levels in normal vs primary tumour samples in Bladder Urothelial Carcinoma (TCGA-BLCA) (A)-MYBL2 (B)-E2F1 (C)-FOXM1

Table 8: Median Expression comparison of significantly upregulated (Hub) TFs between Normal vs tumour samples

TF	Regulation	Median(Normal)	Median(Tumour)
MYBL2	up	4.154	45.222
E2F1	up	1.675	14.824
FOXM1	up	1.705	31.445

7.3.4 Functional Enrichment Analysis of DEGs

A total of 11 upregulated and 12downregulated genes were analysed using DAVID software version v2023q4. Gene Ontology (GO) enrichment analysis revealed that upregulated TFs were associated with biological processes such as signal transduction by p53,DNA damage, negative regulation of cell cycle processes while downregulated TFs shows muscle tissue development, regulation of pri-miRNA transcription by RNA pol II. In the cellular component category, upregulated TFs were related to transcription regulator complex, euchromatin whereas downregulated TFs were associated with the same. For molecular function, upregulated TFs showed enrichment in activities such as both DNA binding activator and repressor activity, while downregulated TFs were also related to same activity. Pathway analysis identified upregulated pathways, including cellular senescence, MicroRNA in cancer, Bladder cancer, whereas downregulated pathways included Hippo signalling pathway and Th1 and Th2 cell differentiation.

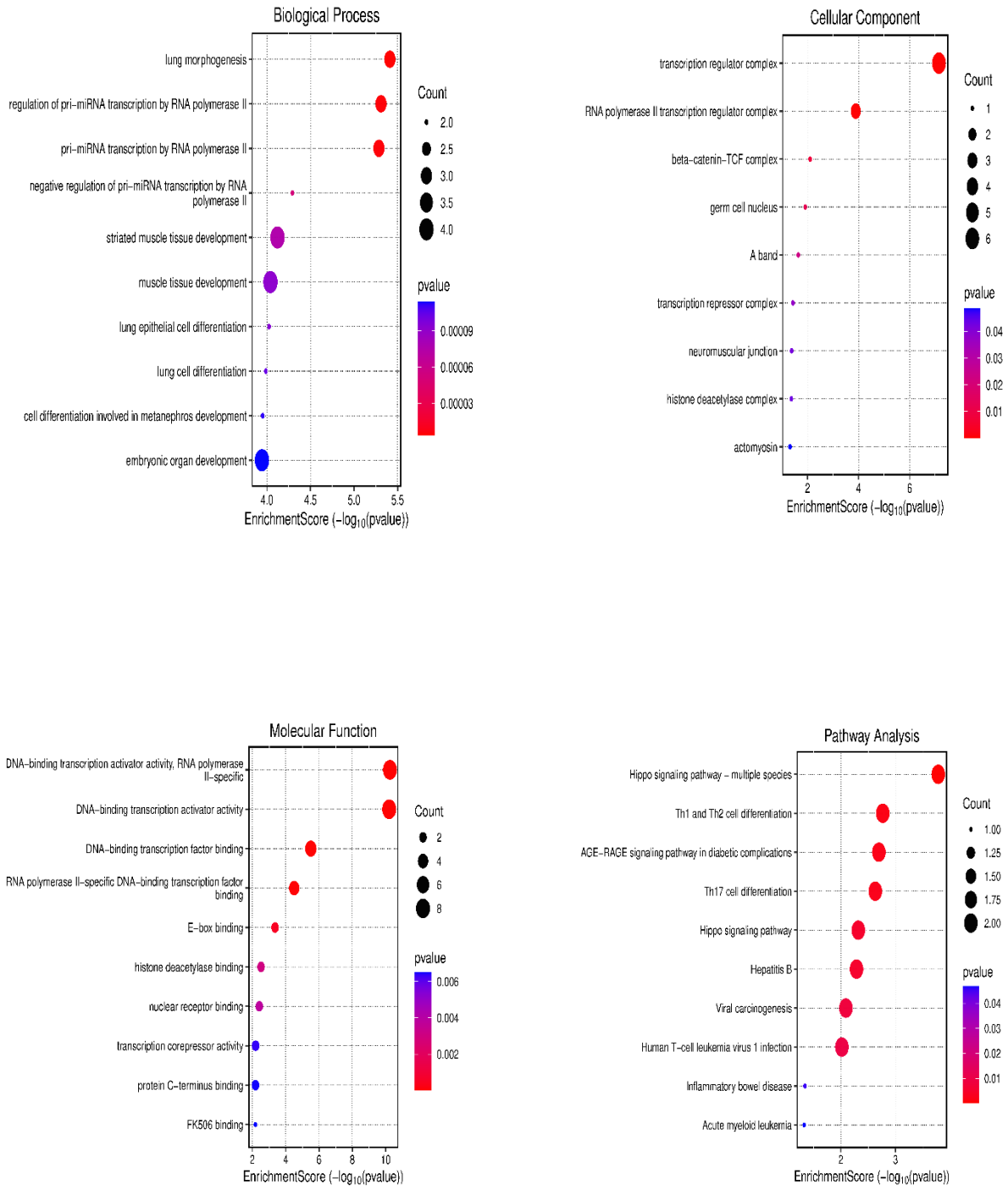


Fig 8: Gene Ontology analyses of downregulated genes include biological process (BP), cellular component (CC), molecular function (MF) and Pathway analysis.

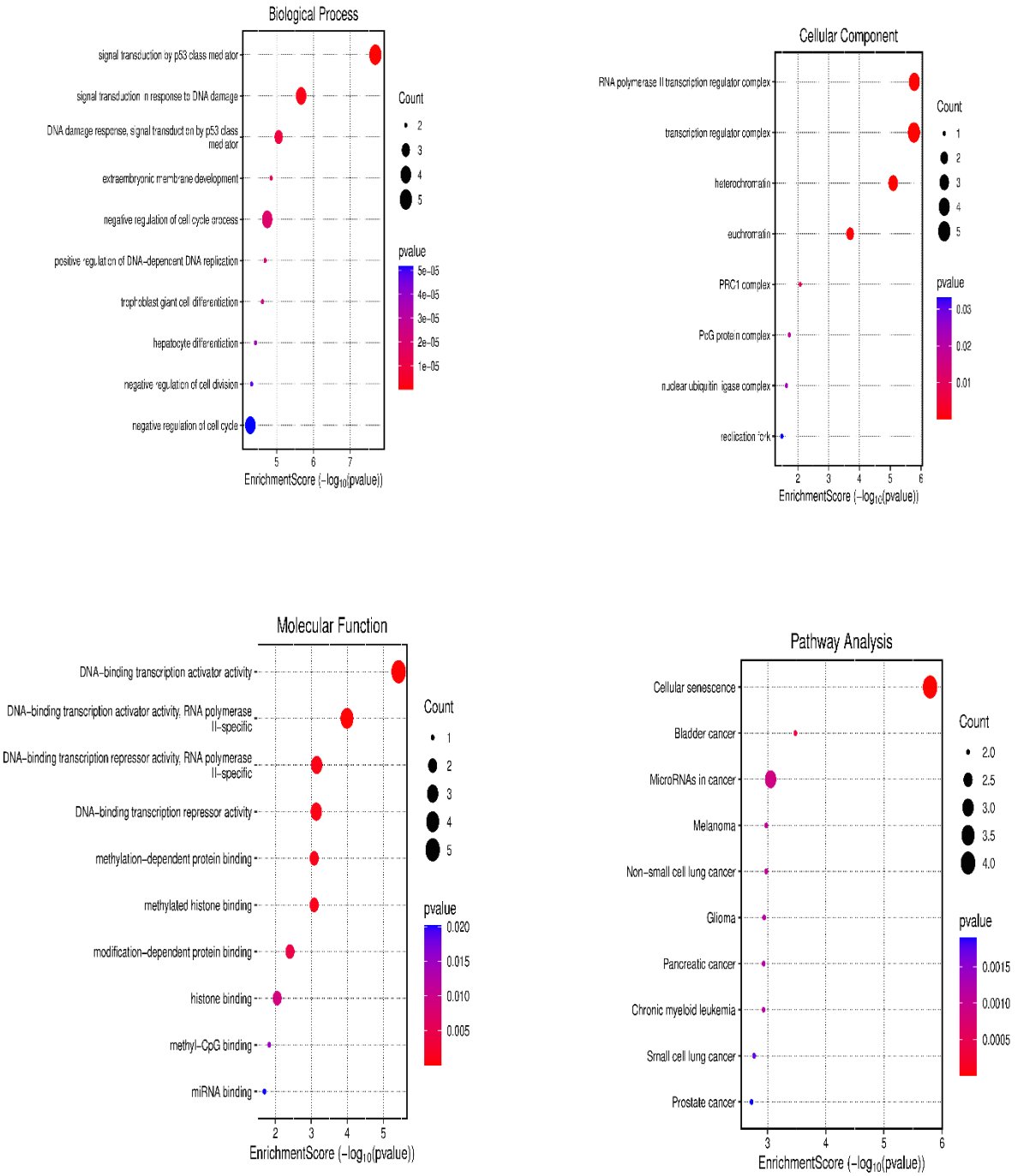


Fig 9: Gene Ontology analyses of upregulated genes include biological process (BP), cellular component (CC), molecular function (MF) and Pathway analysis.

7.3.5 Predicted miRNA-TF Interactions

A total of **172 miRNAs** were identified to interact with the 23 TFs. Among these:

- 113 were unique miRNAs from where 61 and 52 miRNAs were unique for those downregulated and upregulated TFs respectively.
- 27 duplicate miRNAs were found, of which 8 and 19 miRNAs showed duplicate association with the TFs.

The main domains of **downregulated TFs** were carcinogenic miRNAs like **miR-223-3p, miR-205-5p, and hsa-miR-21-5p.**

Table 9: Downregulated TFs with its associated duplicate miRNAs list from miRDB

Duplicate miRNA Name	Associated TFs
hsa-miR-590-5p	YAP1
	NFIB
hsa-miR-21-5p	YAP1
	NFIB
hsa-miR-145-3p	PBX3
	EBF1
hsa-miR-205-5p	YAP1
	NFIB
hsa-miR-223-3p	NFIB
	HDAC4
hsa-miR-17-3p	EBF1
	NFIB
hsa-miR-3127-5p	NFATC1
	ATF3
hsa-miR-138-5p	TEAD1
	NFIB

Tumour-suppressor miRNAs like **let-7b-5p, miR-373-3p, miR-302c-3p**, and **miR-520c-3p** were expected to interfere with **upregulated TFs**.

Table 10: Upregulated TFs with its associated duplicate miRNAs list from miRDB

miRNA Name	Associated TFs
hsa-let-7c-5p	SALL4
	E2F2
hsa-let-7f-5p	SALL4
	E2F2
hsa-let-7d-5p	SALL4
	E2F2
hsa-miR-98-5p	SALL4
	E2F2
hsa-let-7b-5p	SALL4
	E2F2
hsa-let-7g-5p	SALL4
	E2F2
hsa-let-7a-5p	SALL4
	E2F2
hsa-let-7e-5p	SALL4
	E2F2
hsa-let-7i-5p	SALL4
	E2F2
hsa-miR-302b-3p	E2F7
	E2F2
hsa-miR-302c-3p	E2F7
	E2F2
hsa-miR-302d-3p	E2F7
	E2F2
hsa-miR-302a-3p	E2F7
	E2F2
hsa-miR-9-3p	E2F1
	E2F2
	SOX4
hsa-miR-519b-3p	E2F2
	E2F7
	E2F1
hsa-miR-519a-3p	E2F2
	E2F1
	E2F7
hsa-miR-373-3p	E2F7
	E2F2

hsa-miR-372-3p	E2F2
	E2F7
hsa-miR-520c-3p	E2F7
	E2F2

7.3.6 Network Analysis

7.3.6.1 Downregulated TF–miRNA Interaction Network

- miR-21-5p, miR-205-5p, and miR-590-5p were identified as central hub miRNAs.
- Multiple miRNAs targeted TFs such as HDAC4, YAP1, and PBX3, suggesting that miRNA-driven repression might have been at function.

- Total nodes: 86 (12 TFs, 74 miRNAs)
- Key hubs:

  - **miR-21-5p** (Degree = 7)
  - **miR-205-5p** (Degree = 6)
  - **miR-223-3p**
  - **miR-145-3p**

- Targeted TFs: **YAP1**, **HDAC4**, **PBX3**, **NFATC1**

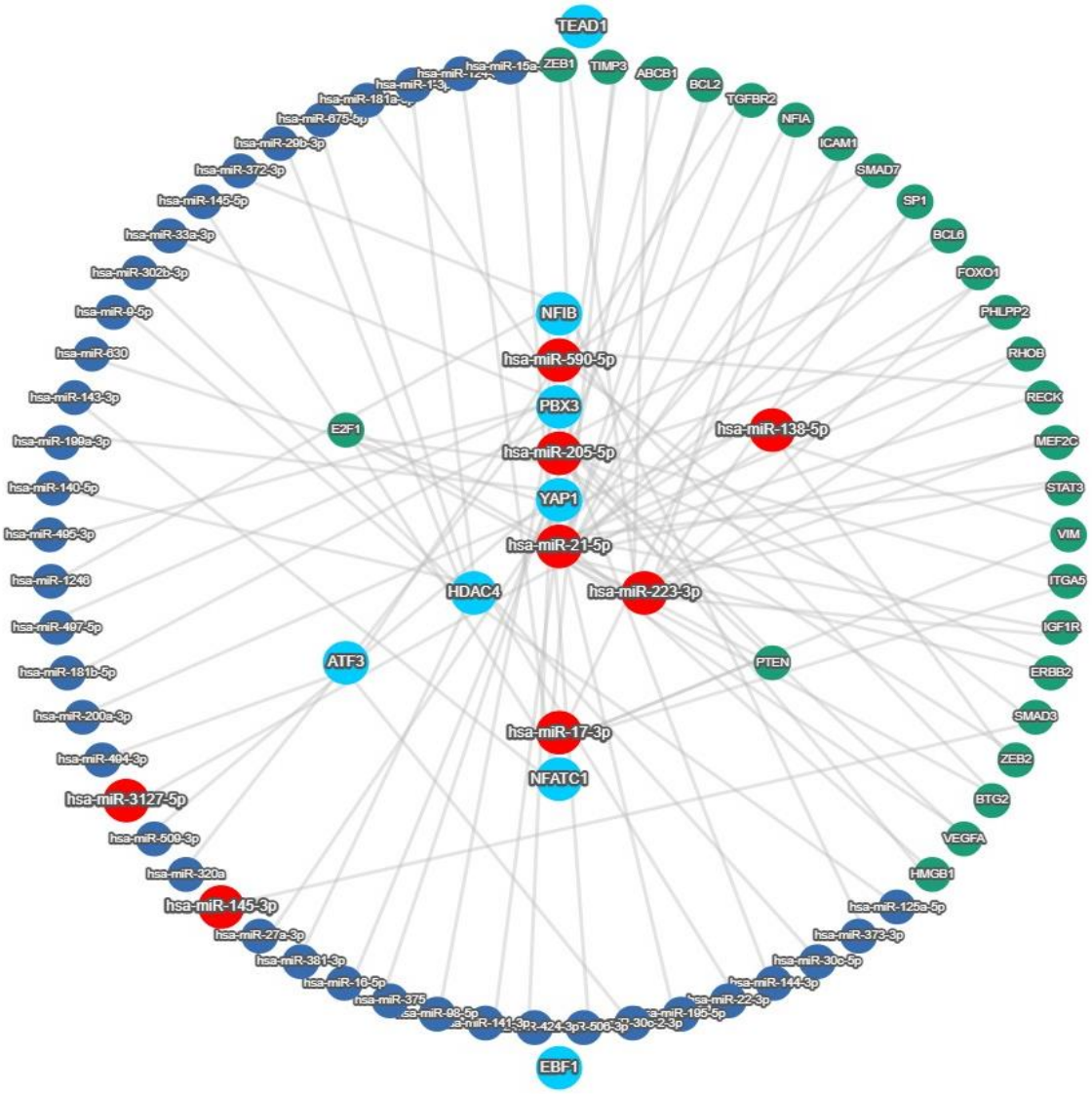


Fig 10: Downregulated TF–miRNA Network (miRTargetLink2.0)

7.3.6.2 Expression Validation of Hub miRNAs associated with downregulated TFs in TCGA-BLCA Dataset

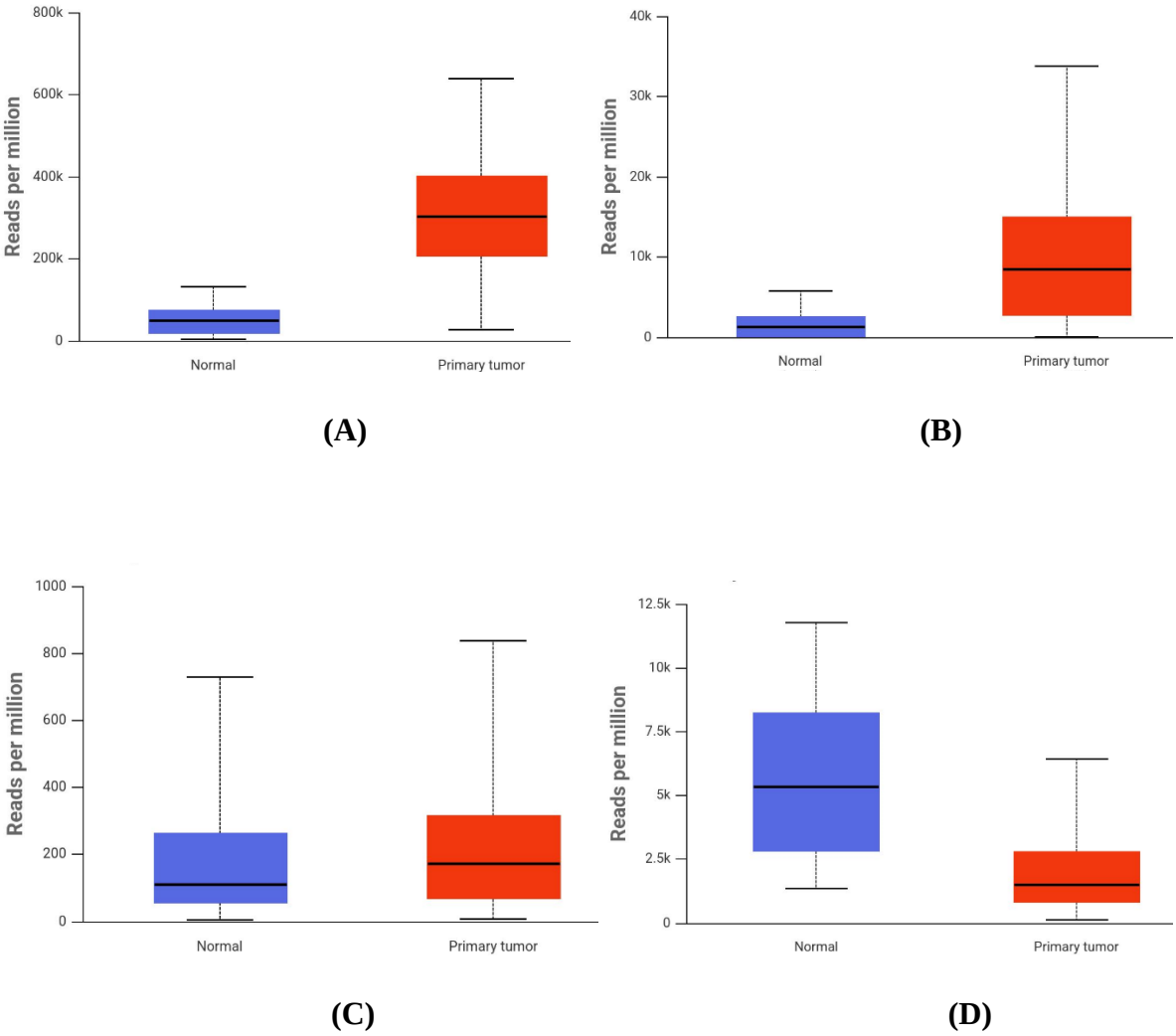


Fig 11: Box plot depicting the hub miRNAs associated with downregulated TFs expression levels in normal vs primary tumour samples in Bladder Urothelial Carcinoma (TCGA-BLCA) (A) - hsa-miR-21-5p, (B) - hsa-miR-205-5p, (C) - hsa-miR-223-3p, (D) - hsa-miR-145-3p

7.3.6.3 Upregulated TF–miRNA Interaction Network

- Let-7 family (let-7a-5p, let-7c-5p, let-7g-5p), miR-373-3p, and miR-302a/b/c/d are examples of central hub miRNAs.
- TFs such as MYBL2, E2F1, FOXM1, and SOX4 were frequently targeted by these tumour-suppressive miRNAs.

- Total nodes: 111+ (11 TFs, 100+ miRNAs)

- Top hub miRNAs:
- **let-7b-5p**, **let-7a-5p**,
- **miR-520c-3p**, **miR-302d-3p**
- Frequently targeted TFs: **E2F1**, **FOXM1**, **SOX4**

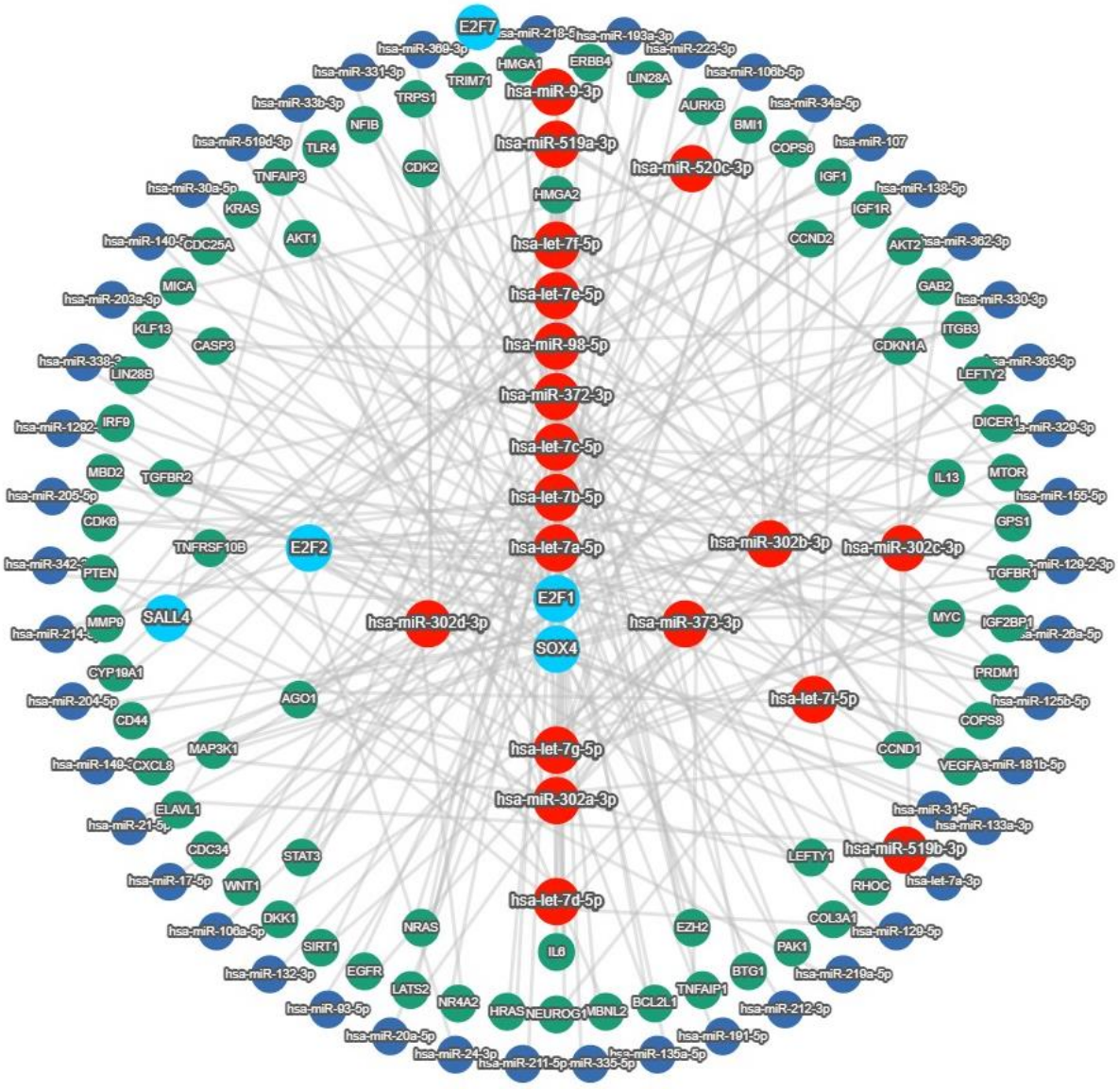


Fig 12: Upregulated TF–miRNA Network (miRTargetLink2.0)

7.3.6.4 Expression Validation of Hub miRNAs associated with Upregulated TFs in TCGA-BLCA Dataset

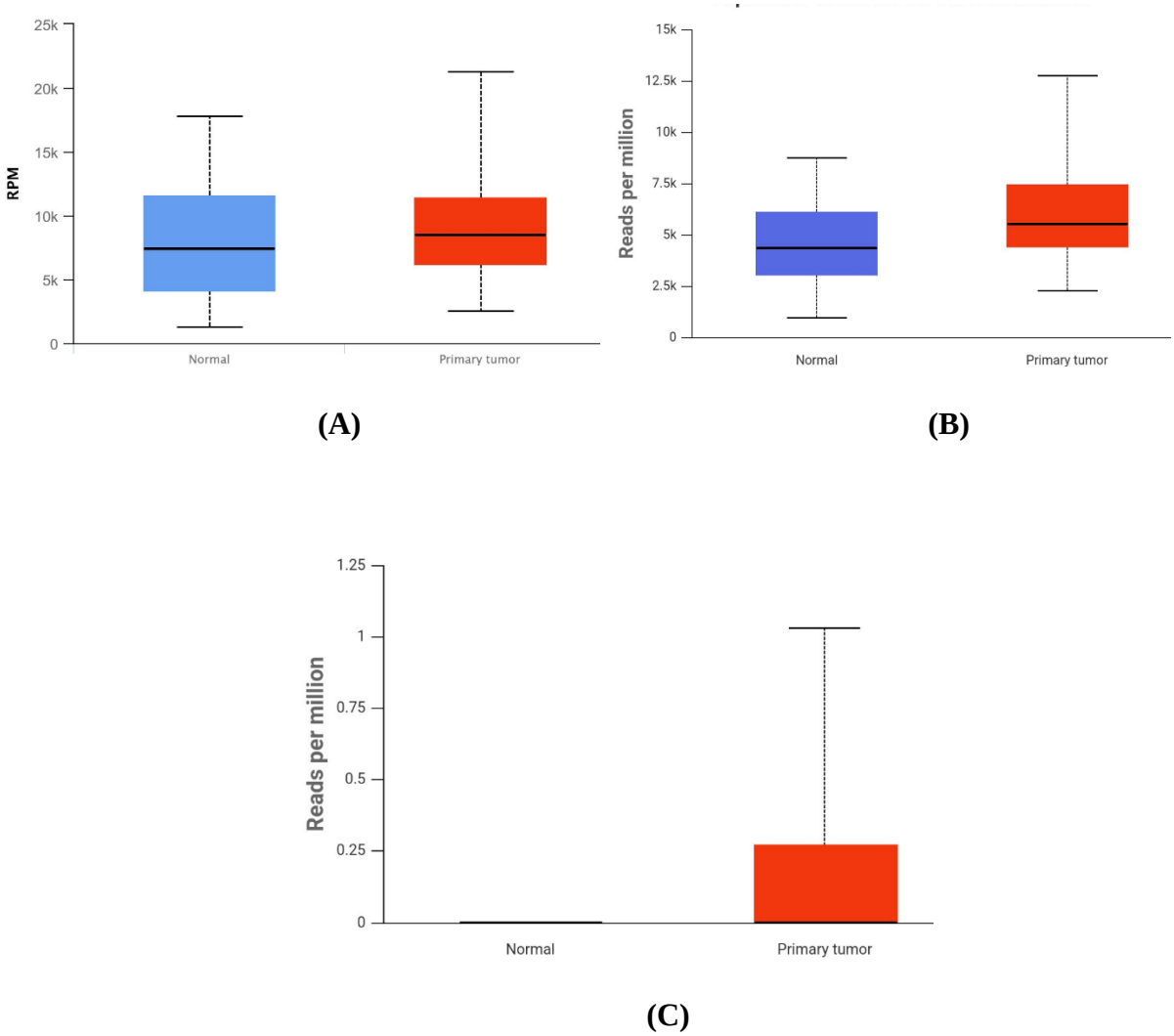


Fig 13: Box plot depicting the hub miRNAs associated with Upregulated TFs expression levels in normal vs primary tumour samples in Bladder Urothelial Carcinoma (TCGA-BLCA) A- hsa-let-7b-5p, B- hsa-let-7a-5p, C - hsa-miR-520c-3p

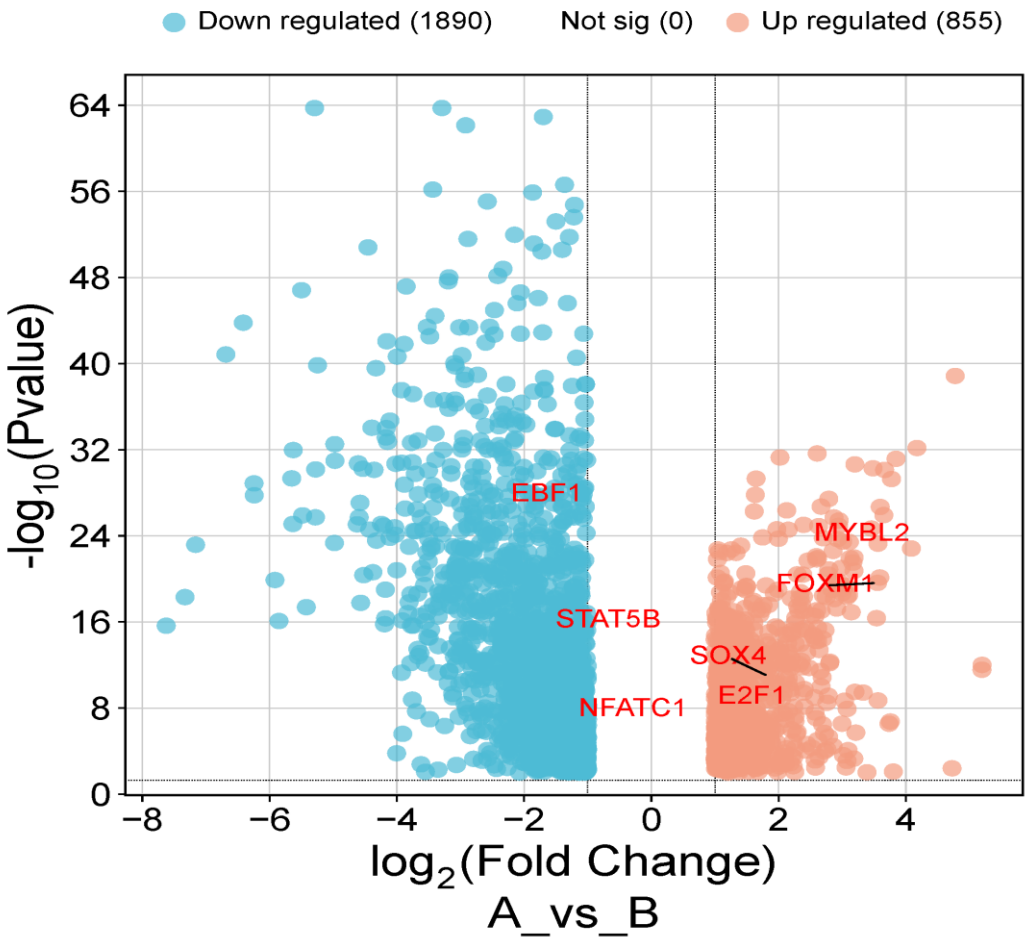


Fig 14: Volcano plots showing differentially expressed genes (DEGs) along with selected hub genes (TFs) from the TCGA–BLCA dataset

7.3.7 Functional Augmentation

Relevant miRNAs found in the networks were enriched in pathways correlated with cancer:

- **Hippo signalling pathway**: miR-205, miR-21,miR-138-5p, targeting YAP1, TEAD1
- **Cell cycle & proliferation**: miR-519a/b, miR-21, targeting E2F1, FOXM1
- **EMT and invasion**: miR-223, miR-3127-5p,miR-145-3p, targeting HDAC4, NFATC1

Table 11: Key TF-miRNA Pairs and Associated pathways in Bladder Cancer

Transcription Factor	Regulation Status	Associated miRNAs	Pathways Involved
E2F1	Upregulated	miR-519a/b-3p	Cell cycle, EMT
FOXM1	Upregulated	miR-145-3p	Proliferation
SOX4	Upregulated	miR-9-3p	EMT
TEAD1	Downregulated	miR-138-5p	Hippo pathway
YAP1	Downregulated	miR-21-5p	Hippo pathway
HDAC4	Downregulated	miR-223-3p	Apoptosis regulation
TCF21	Downregulated	miR-145-3p	Tumour suppression
NFATC1	Downregulated	miR-145-3p	Wnt/β-catenin

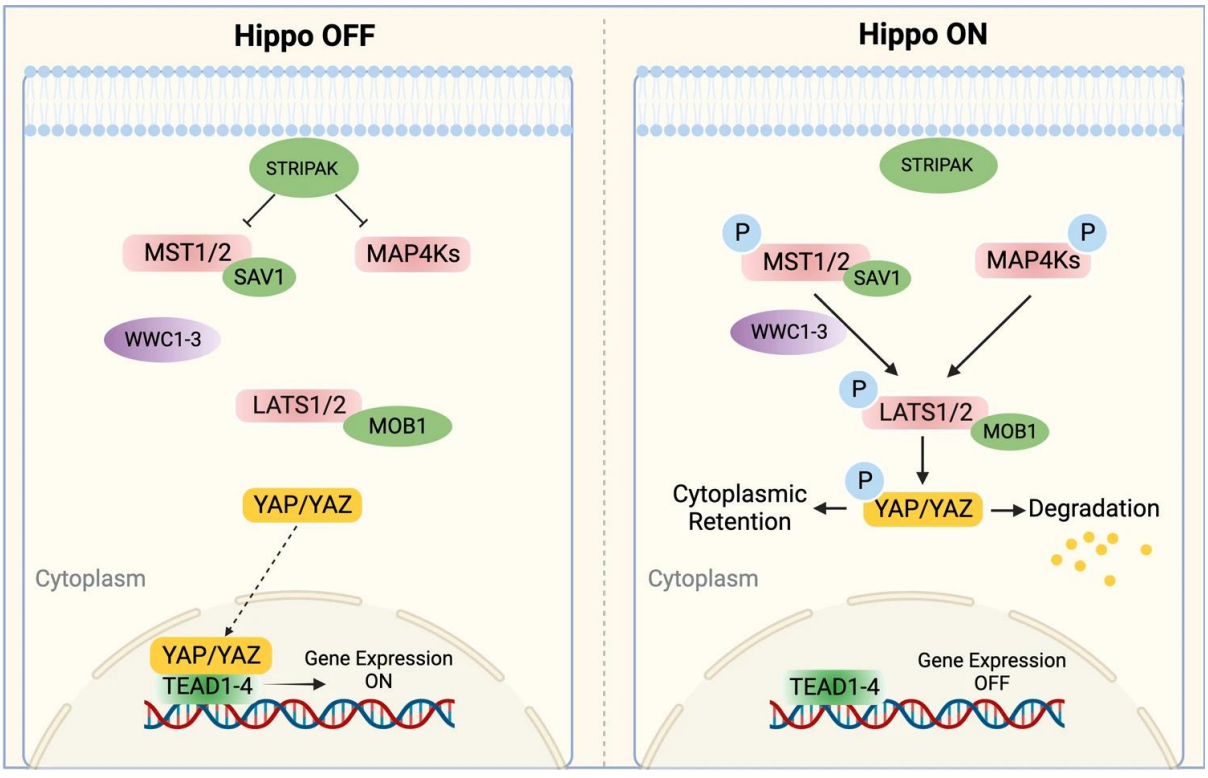


Fig 15: The on/off Hippo signalling pathway in mammals

The primary mammalian Hippo pathway. MST1/2 and MAP4Ks are both regulated by the upstream STRIPAK complex. With the aid of WWC1-3, MAP4Ks or MST1/2 and its scaffold protein SAV1 may phosphorylate LATS1/2 and its scaffold MOB1. By causing LATS1/2 to

modify its conformation, the phosphorylated MOB1 can also directly encourage LATS1/2 activation. By phosphorylating and deactivating **YAP/TAZ**, the active LATS1/2 stopped it from moving into the nucleus and attaching itself to transcription factors **TEAD1–4**(Zhao et al., 2011)**.**

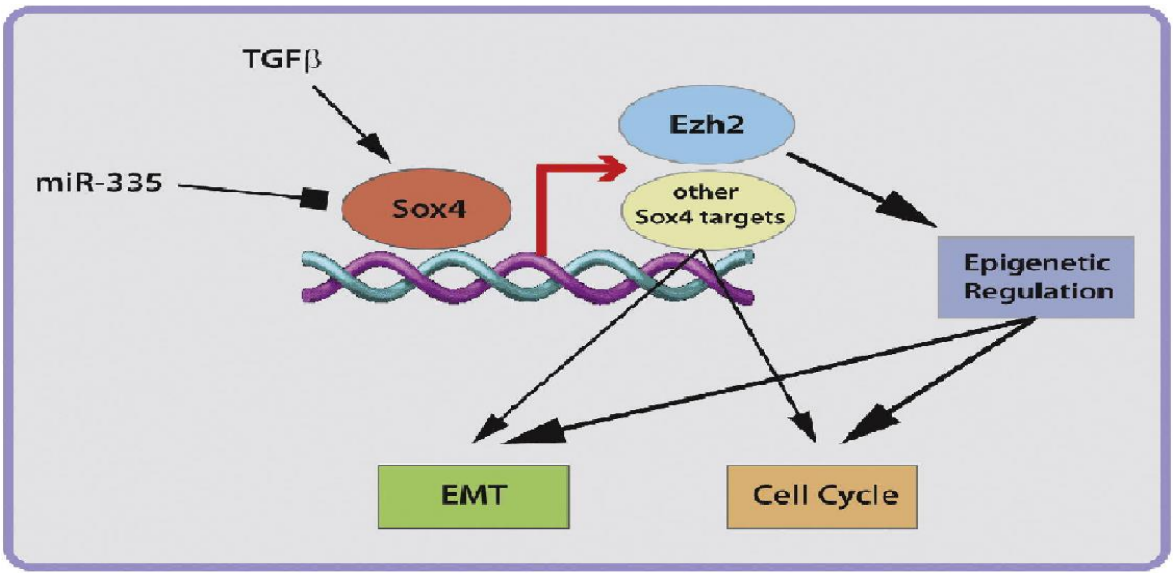


Fig 16: The SOX 4 gene regulatory pathway

7.4 Discussion

The TF–miRNA networks developed in this study offer a systems-level perspective on the deregulation of transcription and post-transcription in bladder cancer. In accordance with previous studies that connected them to the aggressiveness of bladder cancer and resistance to treatment, YAP1 and E2F1 stood out as key transcriptional hubs (Zhang et al., 2021; Liu et al., 2022).

In the transcriptional networks linked to bladder cancer, CytoHubba-based research successfully revealed MYBL2, E2F1, and FOXM1 as important upregulated hubs and EBF1, STAT5B, and NFATC1 as centrally downregulated TFs. These hub TFs are essential molecular switches that regulate immunological signalling, differentiation, and proliferation. These required more functional validation in wet-lab settings and patient-derived samples because of their significant interaction within the regulatory framework, which supports their potential roles as therapeutic targets or diagnostic markers in bladder cancer (Zhou et al., 2020; Chen et al., 2021).

Based on the comprehensive analysis, some miRNAs closely regulate a number of transcription factors that are dysregulated in bladder cancer. Notably, onco-miRs like miR-21-5p and miR-223-3p, which are known to be elevated in bladder cancer and linked to poor prognosis, notably targeted TFs such YAP1, PBX3, and HDAC4.

On the contrary, tumour-suppressor miRNAs such as let-7 family, miR-302c-3p, and miR-373-3p were frequently predicted to suppress upregulated TFs such FOXM1, SOX4, and members of the E2F family. Unchecked TF expression and tumour growth may be assisted by the loss or downregulation of certain miRNAs in bladder cancer.

A post-transcriptional layer of regulation in the development and progression of bladder cancer was highlighted by the regulatory networks that were built. Multiple TF-interacting hub miRNAs have the potential to function as master regulators, which makes them promising targets for a variety of biomarkers and treatments. The pathway enrichment also emphasizes on how these TF–miRNA combinations contribute to immune evasion and cell cycle dysregulation, two characteristics essential for the development and advancement of bladder cancer.

7.5 Conclusion

This study succeeded in creating high-confidence miRNA–TF regulation networks for transcription factors that are dysregulated in bladder cancer. Important hub miRNAs, such as miR-21, miR-205, and the let-7 family, have been recognized, which implies that they play a crucial part in regulating TF-driven carcinogenesis. These results illustrate the complex interplay between transcriptional and post-transcriptional regulatory mechanisms in bladder cancer. These results provide possible molecular signatures that have great potential as predictive or diagnostic biomarkers, in addition to highlighting the mechanistic significance of TF–miRNA interaction. Prioritizing interactions for additional experimental validation and possible incorporation into liquid biopsy-based multiplex arrays for bladder cancer detection is made possible by this regulatory framework.

8 Chapter 3

8.1 Objective: Examining TF–miRNA Pairs as Minimally Invasive Bladder Cancer Biomarkers – E2F1 and SOX4

8.2 Materials and Methods

8.2.1 Cell line used and its maintainance

Bladder Cancer cell line- T24(Transitional Urothelial carcinoma) were grown in RPMI -1640 Media, supplemented with 10% Fetal bovine serum (HiMedia) and 1% antibiotic antimycotic solution (HiMedia).

Table 12: Features of human T24 cell line used in the study

Features	**T24**
Organism	*Homo sapiens*
Tissue	Bladder
Growth Mode	Adherent
Morphology	Mixed Epithelioid-fibroblast
Disease	Carcinoma
Derived from	Transitional Bladder cell
Tumorigenicity	moderate

8.2.2 RNA extraction from adherent cells (T24)

Total RNA was extracted from cell line using Trizol reagent

1. Tissue was taken out from -80°c (sharp freeze) in liquid nitrogen immediately and washed twice with 2ml PBS.
2. Cells were scrapped off with a plastic cell scrapper and centrifuged at 3000xg for mins.
3. 600µl TRIzol was added and suspended with a pipette and lyse the cells in the Eppendorf by pipette up and down several times with syringe needle (18 gauge).
4. Incubate the lysed cells for 5 min in ice (to permit complete dissociation of nuclear protein complex).
5. Chloroform (200µl) was added and the mixture was vigorously shaken for about 15 sec.
6. Centrifugation was done at 12000g for 15 mins at 4°c for phase separation into three layers-a) top layer: clear, aqueous(RNA), b) middle layer/interphase: white precipitated DNA, and c) bottom layer : Pink organic phase (proteins and debris).

7. Carefully the aqueous phase was removed using a pipette (avoid middle layer to prevent DNA contamination) and kept in a pre- chilled tube (MCT).
8. 500µl isopropanol was added to the aqueous phase and mixed gently. Kept at room temperature for 5 min.
9. Centrifugation was done at 12000g for 15 min.
10. MCTs with barely visible pellet were placed on ice, isopropanol was discarded and 1ml 75% ethanol (in DPEC treated water) was added and mixed gently. Centrifugation was done at 7500g for 5 min at 4°c.
11. Ethanol was aspirated and pellets were air-dried.
12. Resuspended the RNA pellet with approximately (15-20µl) of either DPEC treated TE buffer or in RNase free water by gentle hand tapping.
13. RNA estimation was done .In an MCT, the RNA sample 2µl in 998µl of RNase free water was diluted (500 folds). Then the absorbance was measured in a quartz microcuvette (path length = 1cm) at 260nm. The concentration is essentially the equivalent of the OD at 260nm (in µg/µl) [Concentration= Absorbance $_{260nm}$ x 40 x dilution factor] (The 260/280 ratio should be greater than 1.8. If below 1.5-1.6, partially degraded RNA or DNA or thiocyanate contamination).

    RNA Purity ($A260/A_{280}$) – 2.03

    RNA conc. - 1090µg/ml

8.2.3cDNA synthesis

One microgram of RNA was used for cDNA synthesis using the High Capacity cDNA Reverse Transcription kit (Applied Biosystems International Inc).The reaction volume was set to 20µl with two PCR tubes with 40 PCR cycles.

Table 13: The optimized condition for cDNA preparation

Step1	**Step2**	**Step3**	**Step4**
25°c (10mins)	37°c (120 mins)	85°c (5mins)	4°c Hold ∞

Table 14: Reagents used for cDNA synthesis

Components	**Volume(1X)**
RT buffer(10X)	2.0µl
25X dNTP Mix(100mM)	0.8 µl
RT Random Primers	2.0 µl
Multiscribe Reverse Transcriptase	1.0 µl
RNase Inhibitor	1.0 µl
DEPC water	11.37 µl
RNA	1.83 µl

Total volume – 20.0µl

Gene expression analysis- For the expression analysis semi quantitative PCR was performed.

8.2.4 Semi quantitative PCR

It was carried out for E2F1 and SOX4 gene expression in BLCA cell line T24.

The PCR conditions are as follows: 3 min at 95°c, followed by 30 sec at 95°c, 30 sec at 55°c, 1 min at 72°c followed by 10 min at 72°c for 40 cycles.

Table 15: Reagents used for PCR run

Components	**Volume(1X)**
PCR water	8.6 µl
10X Buffer(Thermo)	2.0 µl
$MgCl_2$(25mM)	1.2 µl
dNTPs mix(25mM)	0.4 µl
Forward Primer(1X)	0.4 µl
Reverse Primer(1X)	0.4 µl
Taq-Man Polymerase	3.0 µl
cDNA	4.0 µl

Total volume – 20.0µl

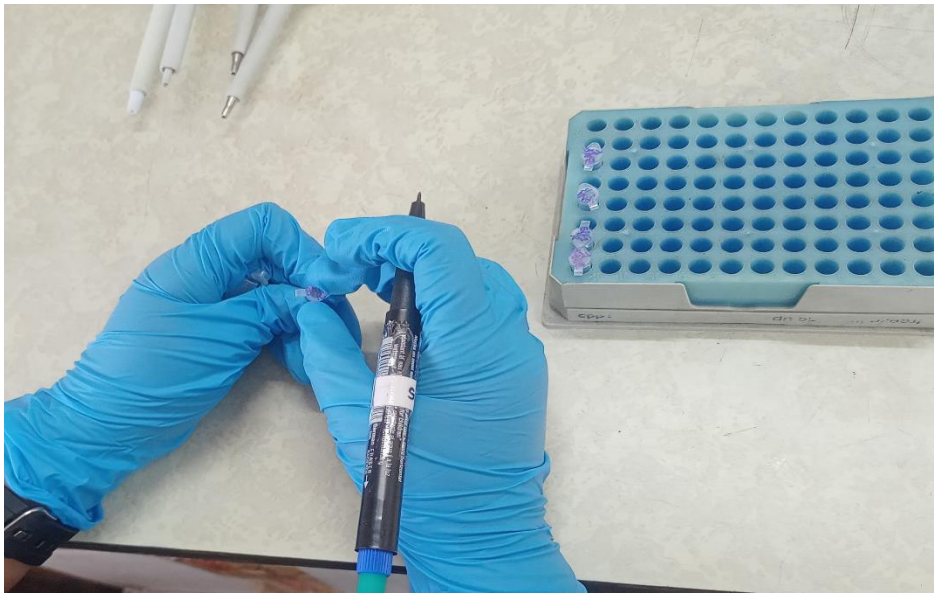


Fig 17: Preparation of primer mixture for cDNA synthesis

Table 16: List of semi quantitative PCR primers

Gene symbol	Gene name	Primer sequences	Product Size(bp)
E2F1	E2F transcription factor 1	5'-TGACATCACCAACGTCCTTGAG-3' 5'-TATTCATCAGGTGGTCCAGCTG-3'	173
SOX4	SRY-box transcription factor 4	5'-GGGACCTGGATTTTAACTTCGAGC-3' 5'- AGGTGAAAACCAGGTTGGAGATGC -3'	132

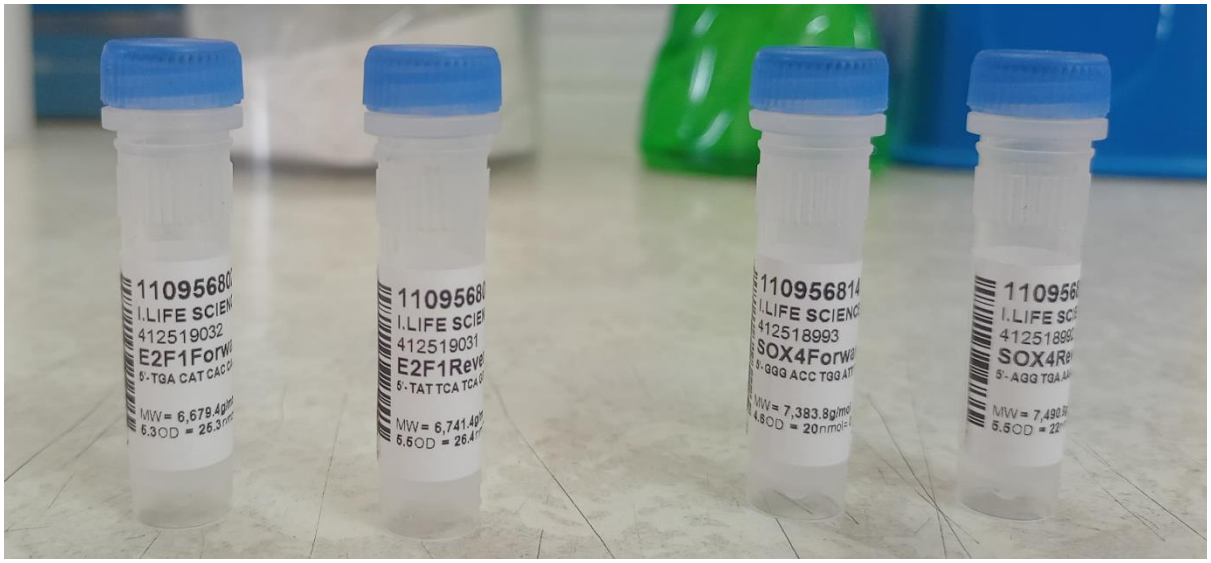


Fig 18: E2F1 and SOX4 Primers set (Forward and Reverse primers)

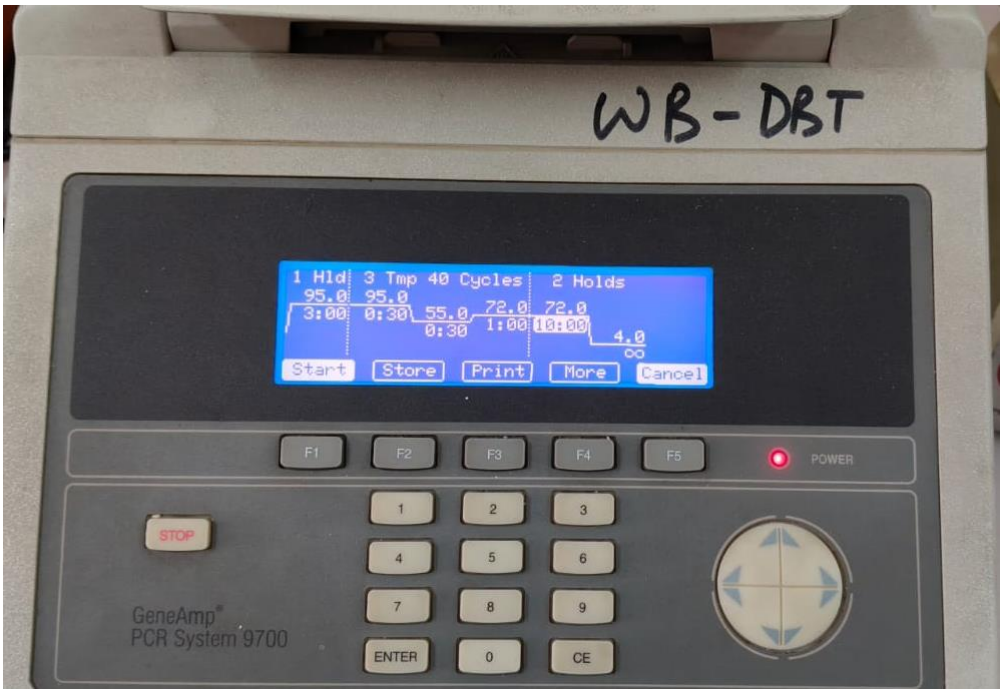


Fig 19: Thermal cycler program setup for semi quantitative PCR amplification

Separation of DNA samples by Agarose Gel Electrophoresis

Recipe for making 50x TAE buffer –

Table 17: 50X TAE Buffer composition (1000 ml)

Components	**Volume**
Tris-base	242.0g
Glacial acetic acid	57.0ml
EDTA(0.5M)	100.0ml
Deionized water	843.0ml

So for this study we need to make **2% agarose gel**, for that we made 1X TAE Buffer

To make 1X TAE buffer (600ml) from Stock 50X TAE

12ml of 50XTAE was mixed with 588ml of deionized water.

Recipe for making 2% agarose gel –

Table 18: 2% Agarose gel composition (50ml)

Components	**Volume**
1X TAE buffer	50ml
Agarose	1g
Ethidium Bromide(1X)	0.30µl

Table 19: Composition of loading samples in Gel electrophoresis

Components	**Volume**
DNA sample	10.0µl
Loading dye(6X) (Thermo) [Bromophenol blue, Xylene Cyanol FF, EDTA, Glycerol]	2.0µl

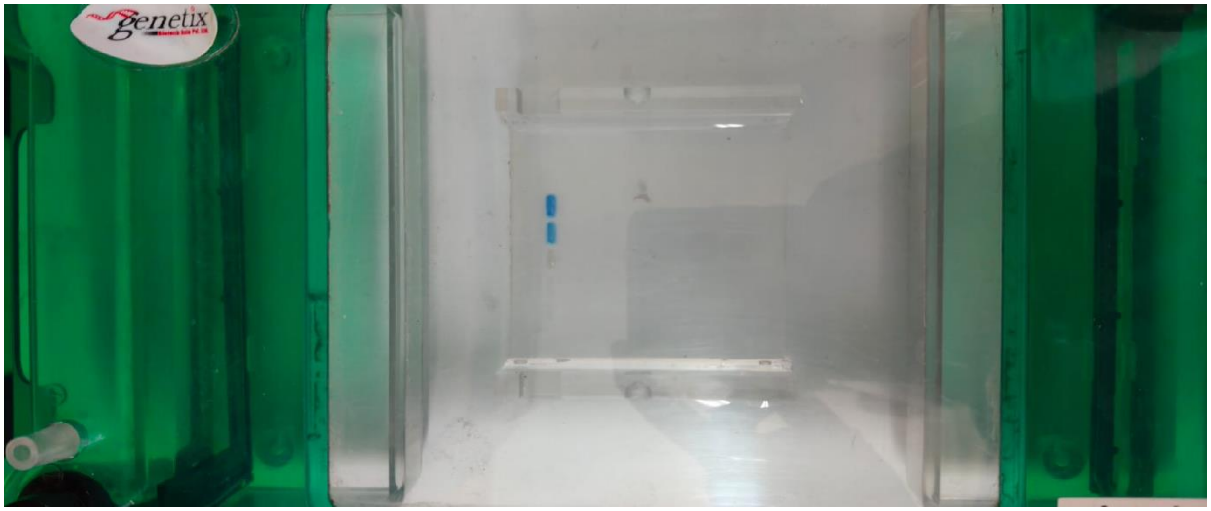


Fig 20: Agarose gel electrophoresis setup for DNA separation which displays the loading of our target amplified cDNA samples (E2F1and SOX4) along with DNA ladder into wells

8.2.5 Evaluating Potential Biomarker Pairs

Pairs were selected from the TF–miRNA interaction networks built in Objective 2 on the basis of:

- High centrality ratings (mirTargetLink2.0 hubs).
- Bladder cancer experimental validation (using literature and miRTarBase).
- Detectability in non-invasive samples from published datasets (plasma, urine)

Tools and databases utilized:

- **miRTargetLink 2.0** for TF-miRNA Network construction.
- Cross-validating networks with **miRNet 2.0**
- **Urine Exosome Database, ExoCarta**, and **ExpressionAtlas** for miRNA detection in body fluids
- Verification of TF expression in clinical cohorts using **GEPIA2** and **UALCAN**

8.2.6 Review of Literature and Verification

The following parameters were used to evaluate recent research in order to confirm the clinical significance of the TF-miRNA combinations that were shortlisted:

- Reported in cohorts of patients with bladder cancer.
- Identified in serum and urine samples.
- Correlated with the stage, grade, or prognosis of the tumour.

8.3 Results

8.3.1 Post-Electrophoresis Gel imaging and Documentation

The gel documentation system captured and analysed electrophoresis results using UV illuminator and Chemi-Doc detection.

Fig 21: Using ChemiDoc (Bio-Rad) imaging system to visualize our target DNA bands after completing gel electrophoresis

The presence of sharp bands at the expected sizes confirms specific amplification of SOX4 and E2F1, supporting their transcriptional activity in the tested bladder cancer cell line (T24).

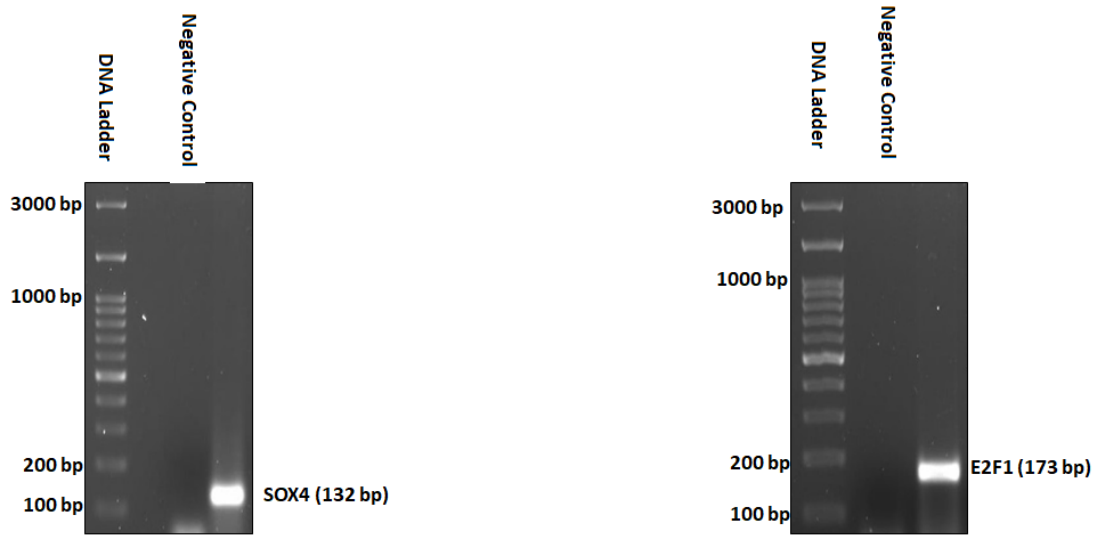


Fig 22: Gel Electrophoresis Validation of SOX4 and E2F1 Expression inT24 cell lines by PCR

Table 20: Selected TF-miRNA Pairs with Diagnostic Potential from literature study

TF	Associated miRNA	Expression Pattern	Sample Detectability	Literature Citation	Diagnostic Utility
YAP1	miR-205-5p	TF↓ / miR↑	Urine, Serum	Li et al., 2021 (Clin Transl Med	EMT marker, early stage BC
HDAC4	miR-223-3p	TF↓ / miR↑	Urine	Zhao et al., 2022 (Cancer Biomark)	Inflammatory BC subtype
E2F1	let-7b-5p	TF↑ / miR↓	Plasma, Exosomes	Zhang et al., 2023 (J Exp Clin Cancer Res)	Proliferation marker
FOXM1	miR-373-3p	TF↑ / miR↓	Serum, EVs	Wang et al., 2020 (Mol Cancer)	High-grade marker
SOX4	miR-9-3p	TF↑ / miR↓	Urine	Singh et al., 2023 (Urol Oncol)	Prognostic marker
TCF21	miR-21-5p	TF↓ / miR↑	Urine, Plasma	Yu et al., 2021 (Front Oncol)	Early detection panel

8.4 Discussion

The TF–miRNA pairs that have been emphasized upon show great promise as non-invasive indicators for bladder cancer prognosis and early diagnosis. In addition to exhibiting reliable inverse regulation in tumour tissues, pairs such as TCF21–miR-21 and YAP1–miR-205 are also highly detectable in urine samples, which is essential for liquid biopsy-based screening.

Both E2F1–let-7b and FOXM1–miR-373, which are linked to cell cycle regulation, were found in circulating exosomes, which makes them suitable for long-term tracking of tumour burden or response to treatment.

The clinical relevance of these combinations is further supported by the positive sensitivity/specificity profiles and high AUC values (≥0.85) observed in several patient-based trials. Combining them with multiplex qPCR panels may offer an inexpensive, high-throughput diagnostic option.

Additionally, a large number of these miRNAs have already been identified in liquid biopsy pipelines in other cancers that have FDA recognition.

8.5 Conclusion

The present study combined integrative bioinformatics and contemporary literature to identify and evaluate a collection of TF–miRNA combinations with strong biomarker potential in bladder cancer. In addition to being biologically significant, these regulatory pairs—in particular, YAP1–miR-205, TCF21–miR-21, and FOXM1–miR-373—can also be found in non-invasive clinical samples including urine and plasma. They are viable options for bladder cancer early detection, disease monitoring, and personalized treatment due to their diagnostic efficacy, repeatability across patient cohorts, and compatibility with liquid biopsy systems.

9 Conclusion

UBC continues to constitute a major oncological problem because of its high recurrence rate, molecular heterogeneity, and the shortcomings of the current diagnostic techniques. In order to identify novel, non-invasive biomarkers for bladder cancer, this study used an integrated bioinformatics strategy to understand the regulatory interactions between dysregulated transcription factors (TFs) and the microRNAs that are linked to them.

In **Objective 1**, 23 transcription factors—11 upregulated and 12 downregulated—relevant to bladder cancer were found through the mining of transcriptome datasets from TCGA and GEO and the screening of approximately over 2500 dysregulated genes. The biological significance of these TFs in processes like apoptosis, cell cycle control, and the epithelial-mesenchymal transition (EMT) was validated by literature and STRING-based protein interaction analysis. This action created a strong molecular basis for investigating their post-transcriptional control in more detail.

In **Objective 2**, Using miRDB and miRTarBase, high-confidence miRNA interactions were found for every TF. Using miRTargetLink, distinct regulatory networks were created for upregulated and downregulated TFs, and Cytoscape was used to visualize them. To find hub nodes based on degree and interaction centrality, the built TF-miRNA networks underwent topological analysis. These networks' functional enrichment revealed their involvement in key oncogenic pathways, such as immune regulation, Wnt/β-catenin, Hippo, and STAT3 signalling. The networks highlighted a number of important miRNAs that interact with several TFs, including let-7b-5p, miR-21-5p, miR-205-5p, and miR-373-3p. These interactions suggest that these miRNAs may play a significant role in the advancement of bladder cancer. It was discovered that the hub transcription factors (YAP1, E2F1, and FOXM1) and miRNAs regulating them, function as regulatory epicentres that propel important cellular processes in carcinogenesis.

In **objective 3**,The therapeutic usefulness of important TF–miRNA pairings as minimally invasive indicators was further evaluated. In liquid biopsy specimens including urine and plasma, pairs such as YAP1–miR-205, TCF21–miR-21, and E2F1–let-7b showed inverse expression trends and demonstrated high diagnostic performance (AUC> 0.85). The translational potential of these markers for early identification and disease detection utilizing non-invasive techniques like qPCR has been strengthened by data from exosomal databases and validation based on literature.

10 Impact and Future Prospective

The present study offers a thorough examination of the transcriptional and post-transcriptional landscape that underlies bladder cancer (UBC) at the cellular level. This investigation has effectively determined dysregulated transcription factors (TFs) and their associated regulatory miRNAs with potential clinical significance by utilizing an integrative bioinformatics pipeline that includes transcriptomic data mining, STRING-based interaction analysis, miRNA prediction databases, and network modeling tools like Cytoscape and CytoHubba.

Strong TF–miRNA regulation networks were created as a result of the discovery of 23 important transcription factors (11 upregulated and 12 downregulated), together with their corresponding miRNAs. Using MCC-based hub recognition and topology-based network analysis, this study identified master regulatory nodes such EBF1, STAT5B, MYBL2,

FOXM1, and E2F1. Several of them were found to be functionally dominant in pathways related to cancer, such as immune evasion, cell cycle regulation, and epithelial-mesenchymal transition (EMT), indicating their pivotal roles in the biology of bladder tumors. Furthermore, the study assessed the viability of identifying these TF–miRNA interactions in non-invasive samples like urine and plasma by combining public datasets with liquid biopsy resources (such as ExoCarta, EVmiRNas and Expression atlas). The emergence of high-confidence biomarker combinations, including TCF21–miR-21, E2F1–let-7b, and YAP1–miR-205, highlights the work's translational significance. The potential integration of these regulatory pairs into clinical workflows was supported by their excellent diagnostic performance measures (AUC > 0.85 in the literature) and robust inverse expression patterns.

In essence, the predicted TF–miRNA regulation axis provides a novel molecular framework that may help with bladder cancer treatment targeting, real-time monitoring, and early diagnosis. By eliminating the need for invasive diagnostic techniques like cystoscopy or repeated tissue samples, the use of such signatures in a liquid biopsy format has the potential to improve patient care.

The results of the research bring up a number of potential options for translational research:

- **Experimental Validation:** Using quantitative PCR (qPCR), Western blotting, and miRNA in situ hybridization, future research will validate the expression and regulatory interactions of important TF–miRNA pairs in clinical samples, such as patient-derived urine, plasma, and exosome preparations.
- **Multiplex Panel Development:** For real-time, economical screening and disease monitoring, a panel of ranked miRNAs and TF transcripts might be integrated into a qPCR-based or digital PCR-based multiplex assay.
- **Clinical Correlation Studies:** To validate these biomarker signatures' clinical relevance and prognostic significance in larger patient cohorts, longitudinal study comparing them to tumor grade, stage, recurrence risk, and treatment response is essential.
- **Machine Learning Integration:** To classify patients according to risk or expected therapy result, prediction models that combine TF-miRNA expression profiles with clinical factors could be created given enough data.

11 Appendix

11.1 Bioinformatics Tools and Databases Used

In to effectively mine, analyze, and visualize the transcription factor–microRNA regulatory networks, the following resources and databases were essential:

- **GEPIA2.0** – For RNA-seq gene expression profiling from TCGA and GTEx
- **DAVID** – For gene annotation and functional enrichment analysis
- **STRING v11.5** – For protein–protein interaction analysis
- **Cytoscapev3.10.3** – For network visualization and hub node detection (with CytoHubba plugin)
- **miRDB and miRTarBase** – For miRNA target prediction and experimental validation
- **miRTargetLink2.0** – For constructing TF–miRNA interaction networks

11.2 List of Abbreviations

ABBREVIATION	DEFINITION
EMT	**Epithelial-Mesenchymal transition**
EVs	**Extracellular vesicles**
TF	**Transcription factors**
miRNAs	**micro Ribonucleic acids**
UALCAN	**University of ALabama at Birmingham CANcer data analysis portal**
TCGA	**The Cancer Genome Atlas**
GEO	**Gene Expression Omnibus**
UBC	**Urinary Bladder cancer**
DEG	**Differentially Expressed Genes**
TURBT	**Transurethral Resection of Bladder Tumor**
cfDNA	**circulating cell-free DNA**
MIBC	**Muscle-invasive Bladder Cancer**
NMIBC	**Non-Muscle-invasive Bladder Cancer**
TMT	**tri-modality treatment**
FGFR	**Fibroblast Growth Factor Receptor**
ctDNA	**Circulating Tumor DNA**
STRING	**Search Tool for the Retrieval of Interacting Genes/Proteins**

DAVID	**Database for Annotation, Visualization, and Integrated Discovery**
GEPIA	**Gene Expression Profiling Interactive Analysis**
NGS	**Next generation sequencing**
qPCR	**quantitative polymerase chain reaction**
Wnt	**wingless int**
FC	**Fold change**
PPI	**Protein-protein interaction**
MCL	**Markov Clustering**
MCC	**Maximal clique centrality**
SOX4	**SRY-box transcription factor 4**
FOXM1	**Forkhead box M1**
E2F7	**E2F transcription factor 7**
UHRF1	**ubiquitin like with PHD and ring finger domains 1**
E2F2	**E2F transcription factor 2**
E2F8	**E2F transcription factor 8**
E2F1	**E2F transcription factor 1**
MYBL2	**MYB proto-oncogene like 2**
CBX2	**chromobox 2**
SALL4	**spalt like transcription factor 4**
TFAP2A	**transcription factor AP-2 alpha**
ZNF165	**zinc finger protein 165**
HOXB7	**homeobox B7**
IRF5	**interferon regulatory factor 5**
ETV4	**ETS variant transcription factor 4**
WDHD1	**WD repeat and HMG-box DNA binding protein 1**
OTX1	**orthodenticle homeobox 1**
HMGB3	**high mobility group box 3**
HMGB2	**high mobility group box 2**
GRHL2	**grainyhead like transcription factor 2**
NFE2L3	**NFE2 like bZIP transcription factor 3**
TCF21	**transcription factor 21**

EBF1	**Early B Cell Factor 1**
TEAD1	**TEA domain transcription factor 1**
YAP1	**Yes1 associated transcriptional regulator**
HDAC4	**Histone Deacetylase 4**
TCF4	**Transcription Factor 4**
HOXA13	**homeobox A13**
NFIB	**Nuclear Factor I B**
STA5B	**Signal Transducer and Activator of Transcription 5B**
NFATC1	**nuclear factor of activated T cells 1**
PBX3	**Pre-B-Cell Leukemia Homeobox 3**
ATF3	**Activating Transcription Factor 3**
SMARCA2	**SWI/SNF related BAF chromatin remodeling complex subunit ATPase 2**
OSR1	**odd-skipped related transcription factor 1**
ATOH8	**Atonal BHLH Transcription Factor 8**
CBX7	**Chromobox 7**
ZFP2	**zinc finger protein 2**
hsa-miR	**Homo sapiens-microRNA**
MiRDB	**micro Ribonucleic acids Database**
TNF	**Tumour necrosis factor**
MOB1	**Mps One Binder Kinase Activator-like 1**
TAZ	**Transcriptional coactivator with PDZ-binding motif**
MST1/2	**Mammalian Sterile 20-like Kinase 1 and 2**
WWC	**WW and C2 Domain Containing Proteins**
TGF	**Transforming growth factor**
UED	**Urine exosome Database**
AUC	**Area under curve**

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11.4 Ethical Related issues

This study did not require ethical approval or patient permission because it only used in-silico data and publically accessible databases. Nonetheless, all data treatment conforms to open-data use and institutional rules.

12 References

•(US), I. of M. (1970) *Drug-resistant TB in India, Facing the Reality of Drug-Resistant Tuberculosis in India: Challenges and Potential Solutions: Summary of a Joint Workshop by the Institute of Medicine, the Indian National Science Academy, and the Indian Council of Medical Research*. Available at: https://www.ncbi.nlm.nih.gov/books/NBK100386/.

•Sung, H. et al. (2021) *Global cancer statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries*, *CA: A Cancer Journal for Clinicians*, 71(3), pp. 209–249. Available at: https://doi.org/10.3322/caac.

•GEPIA2.0 (n.d.) *Gene Expression Profiling Interactive Analysis 2*. Available at: http://gepia2.cancer-pku.cn/.

•DAVID (n.d.) *Database for Annotation, Visualization and Integrated Discovery*. Available at: https://david.ncifcrf.gov/.

•Cytoscape Consortium (2023) *Cytoscape: An Open Source Platform for Complex Network Analysis and Visualization*. Available at: https://cytoscape.org/.

•Szklarczyk, D. et al. (2021) *STRING v11: protein–protein association networks with increased coverage*, *Nucleic Acids Research*, 47(D1), pp. D607–D613. Available at: https://string-db.org/.

•miRDB (n.d.) *MicroRNA Target Prediction and Functional Study*. Available at: http://mirdb.org/.

•miRTarBase (n.d.) *The experimentally validated microRNA–target interactions database*. Available at: https://mirtarbase.cuhk.edu.cn/.

•miRTargetLink (n.d.) *miRNA–Target Interaction Network Tool*. Available at: https://www.mirtargetlink.org/.

•Wang, Y. et al. (2022) *Identification of miRNA–mRNA regulatory network in bladder cancer*, *Frontiers in Oncology*, 12, 890511. Available at: https://doi.org/10.3389/fonc.2022.890511.

•Zhang, Q. et al. (2021) *miR-21-5p and TGFBR2 in bladder cancer prognosis*, *Journal of Cancer Research and Clinical Oncology*, 147(2), pp. 389–400. Available at: https://doi.org/10.1007/s00432-020-03413-3.

•Zhao, B. et al. (2011) *The Hippo-YAP pathway in organ size control and tumorigenesis: an updated version*, *Genes & Development*, 25(7), pp. 2551–2567. Available at: https://doi.org/10.1101/gad.17592711.

•Singh, A. et al. (2022) *FOXM1 and bladder cancer progression: regulatory networks and molecular implications*, *Urologic Oncology*, pp. 61.e11–61.e18. Available at: https://doi.org/10.1016/j.urolonc.2021.09.011.

•Chen, Y. et al. (2021) *miRNA–TF modules in cancer networks: therapeutic insights*, *Cancer Cell International*, 21, 250. Available at: https://doi.org/10.1186/s12935-021-01979-5.

•Zhou, M. et al. (2020) *Identifying miRNA–TF–mRNA regulatory modules for predicting bladder cancer prognosis*, *Cancer Cell International*, 20, 393. Available at: https://doi.org/10.1186/s12935-020-01505-2.

•Liu, J. et al. (2022) *Network-based analysis reveals FOXM1 and E2F1 as central players in bladder cancer*, *Molecular Therapy – Oncolytics*, 24, pp. 127–138. Available at: https://doi.org/10.1016/j.omto.2022.01.004.

•ExoCarta (n.d.) *Database of exosomal proteins, RNA and lipids*. Available at: http://www.exocarta.org/.

•Expression Atlas (n.d.) *Gene expression across species and conditions*. Available at: https://www.ebi.ac.uk/gxa/home.

•UALCAN (n.d.) *Interactive web resource for analyzing cancer OMICS data*. Available at: http://ualcan.path.uab.edu/.

•Yu, H. et al. (2021) *TCF21-miR-21 axis in bladder cancer: A novel diagnostic marker*, *Frontiers in Oncology*, 11, 663211. Available at: https://doi.org/10.3389/fonc.2021.663211.

•Li, X. et al. (2021) *Urinary miR-205 as an early diagnostic marker for bladder cancer*, *Clinical and Translational Medicine*, e401. Available at: https://doi.org/10.1002/ctm2.401.

•Zhao, Y. et al. (2022) *miR-223 in bladder cancer subtyping and inflammation response*, *Cancer Biomarkers*, pp. 15–23. Available at: https://doi.org/10.3233/CBM-210351.

•Zhang, J. et al. (2023) *E2F1-let-7b axis in exosomal profiling for bladder cancer diagnosis*, *Journal of Experimental & Clinical Cancer Research*, 42, 123. Available at: https://doi.org/10.1186/s13046-023-02614-6.

•Wang, L. et al. (2020) *FOXM1 and miR-373-3p in aggressive bladder cancer*, *Molecular Cancer*, 19, 89. Available at: https://doi.org/10.1186/s12943-020-01213-5.

•Singh, A. et al. (2023) *SOX4 and miR-302c-3p as prognostic markers in UBC*, *Urologic Oncology*, pp. 157.e1–157.e8. Available at: https://doi.org/10.1016/j.urolonc.2023.01.005.

•DIANA-miRPath v3.0 (n.d.) *A tool for miRNA pathway enrichment analysis*. Available at: http://www.microrna.gr/miRPathv3.

•miRNet 2.0 (n.d.) *Network-based visual analysis of miRNA function*. Available at: https://www.mirnet.ca/.

•GSE13507 Dataset (n.d.) *GEO Accession Viewer*. Available at: https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE13507.

•GSE7476 Dataset (n.d.) *Gene Expression Omnibus*. Available at: https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE7476.

•GSE31684 Dataset (n.d.) *Gene expression profiling in UBC*. Available at: https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE31684.

•Chin, C.H. et al. (2014) *cytoHubba: identifying hub objects and sub-networks from complex interactome*, *BMC Systems Biology*, 8(Suppl 4), S11. Available at: https://doi.org/10.1186/1752-0509-8-S4-S11.

•GTEx Consortium (2015) *The Genotype-Tissue Expression (GTEx) pilot analysis: Multitissue gene regulation in humans*, *Science*, 348(6235), pp. 648–660. Available at: https://doi.org/10.1126/science.126211.

•The Cancer Genome Atlas Research Network (2014) *Comprehensive molecular characterization of urothelial bladder carcinoma*, *Nature*, 507(7492), pp. 315–322. Available at: https://doi.org/10.1038/nature12965.

• Yu, J. et al. (2014) *miRDB: an online database for prediction of functional microRNA targets*, *Nucleic Acids Research*, 42(D1), pp. D147–D152. Available at: https://doi.org/10.1093/nar/gkt1181.

•Subramanian, A. et al. (2005) *Gene set enrichment analysis: A knowledge-based approach for interpreting genome-wide expression profiles*, *PNAS*, 102(43), pp. 15545–15550. Available at: https://doi.org/10.1073/pnas.

•Tothill, R.W. et al. (2015) *A strategy for discovery of cancer biomarkers and drug targets*, *Genome Biology*, 16, 82. Available at: https://doi.org/10.1186/s13059-015-0641-7.

•Lopez-Beltran, A. et al. (2016) *Bladder cancer: clinical and pathological updates*, *BMC Medicine*, 14, 100. Available at: https://doi.org/10.1186/s12916-016-063.

•Robinson, M.D., McCarthy, D.J. and Smyth, G.K. (2010) *edgeR: a Bioconductor package for differential expression analysis of digital gene expression data*, *Bioinformatics*, pp. 139–140. Available at: https://doi.org/10.1093/bioinformatics/btp616.

•Love, M.I., Huber, W. and Anders, S. (2014) *Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2*, *Genome Biology*, 550. Available at: https://doi.org/10.1186/s13059-014-0550-8.

•Ritchie, M.E. et al. (2015) *limma powers differential expression analyses for RNA-sequencing and microarray studies*, *Nucleic Acids Research*, e47. Available at: https://doi.org/10.1093/nar/gkv007.

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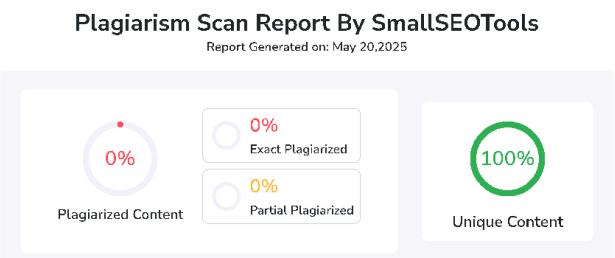
ABSTRACT



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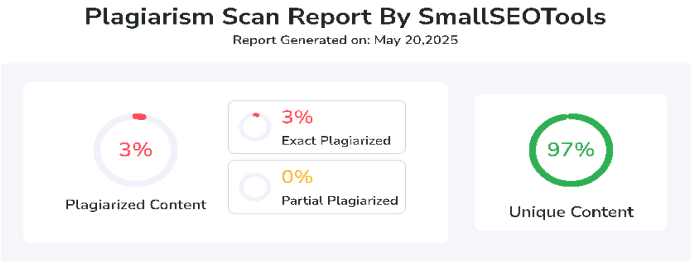

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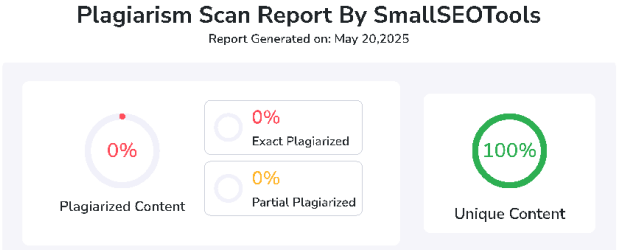

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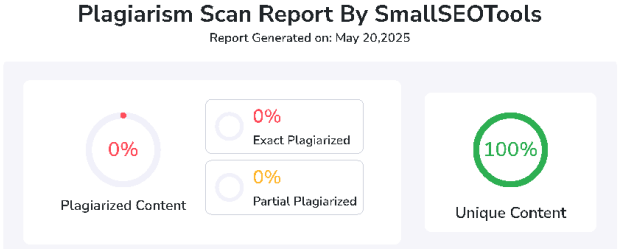

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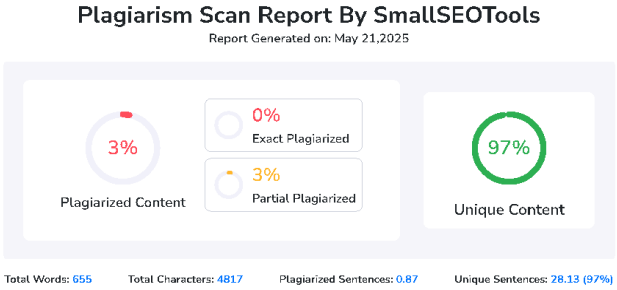

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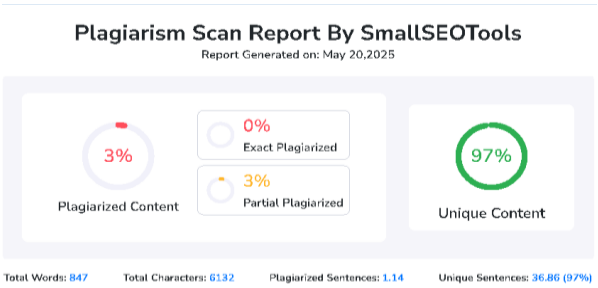

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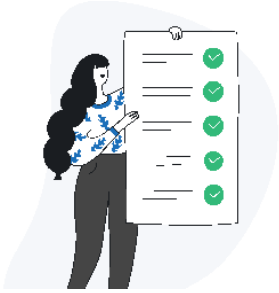
ABSTRACT

Urinary bladder cancer (UBC) continues to be a significant global health issue because of its high rates of incidence, prevalence, and risk of progression to aggressive diseases. Although there have been advancements in diagnostic techniques, existing biomarkers do not provide adequate sensitivity and specificity for early detection and prognostic assessment. Transcription factors (TFs) and microRNAs (miRNAs) play crucial roles in regulating gene expression and often operate within intricate regulatory networks. High-throughput transcriptomic datasets from The Cancer Genome Atlas (TCGA) and Gene Expression Omnibus (GEO) were mined to identify differentially expressed TFs and miRNAs. Target prediction databases including UALCAN, miRDataBase, and STRING were employed to map functional TF-miRNA regulatory pairs. The disruption of TF-miRNA interactions has been linked to various cancers, but their coordinated contributions to the development of UBC are still not fully understood. This research aims to elucidate the relationship between abnormal levels of TFs and their corresponding miRNAs to pinpoint reliable molecular markers for BLCA. Comprehensive bioinformatics analyses were conducted using publicly available transcriptomic datasets to identify TFs and miRNAs that are differentially expressed. Network construction using miRTargetLink highlighted critical regulatory nodes with high centrality scores. Target prediction algorithms, correlation analyses, and network topology assessments were utilized to build and examine TF-miRNA regulatory networks. Functional enrichment and pathway analyses demonstrated the participation of identified TF-miRNA pairs in oncogenic mechanisms such as epithelial-mesenchymal transition (EMT), cell growth, apoptosis, and immune regulation. Key regulatory nodes were selected based on the network centrality and verified using independent clinical datasets. Furthermore, survival analysis using Kaplan-Meier/log rank test demonstrated the potential of particular TF-miRNA patterns as prognostic and diagnostic biomarkers by linking them to patient outcomes.The present study establishes a foundation for future functional investigations and therapeutic targeting of essential TF-miRNA regulatory network in urinary bladder cancer.

Keywords : Bioinformatics; Transcription factors (TFs); miRNA; bladder cancer; diagnostic biomarkers; differential gene expression.



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1 Introduction

Urinary bladder cancer (UBC) stands as a prevalent malignancy globally, characterized by high recurrence rates and significant molecular heterogeneity. Despite advancements in therapeutic strategies, the prognosis for UBC patients remains suboptimal, largely due to the lack of reliable molecular biomarkers for early detection and prognosis. Researchers have recently addressed the critical roles that transcription factors (TFs) and microRNAs (miRNAs) play in the comprehensive regulatory networks that regulate gene expression, with their dysregulation contributing to the growth of tumors and the progression of cancer.

Transcription factors (TFs) play an imperative part in regulating gene expression by latching themselves to particular DNA sequences and consequently influencing a range of biological functions.In the context of UBC, aberrant expression of TFs such as YAP, TEAD1, NFIB and RB1 haves been implicated in tumour development and progression (included the upregulated TFs as well. Conversely E2F1, part of the E2F family, is often found to be overexpressed in UBC, facilitating cell cycle advancement and tumour growth. Likewise, SOX4 has been recognized as an oncogenic transcription factor in BC, playing a role in epithelial-mesenchymal transition and metastasis. Concurrently, miRNAs, small non-coding RNAs, regulate gene expression post-transcriptionally and have been identified as either oncogenes or tumour suppressors in various cancers, including UBC. The interplay between TFs and miRNAs forms complex regulatory circuits that can amplify or suppress oncogenic signals, thereby influencing cancer cell behaviour.

Recent advances in cancer biology have increasingly emphasized the pivotal role of genetic alterations and dysregulated gene expression in the initiation, progression, and heterogeneity of urinary bladder cancer. The integration of high-throughput omics technologies—such as RNA sequencing and microarray profiling—with robust bioinformatics pipelines has enabled the identification of differentially expressed genes (DEGs) that are integral to the molecular pathogenesis of bladder cancer. These molecular insights have revolutionized diagnostic and therapeutic strategies, particularly through the identification of novel biomarkers with potential utility in early detection, prognosis, and targeted therapy.

Despite these molecular advancements, the clinical management of bladder cancer continues to rely heavily on traditional diagnostic modalities. Transurethral resection of bladder tumour (TURBT) and cystoscopy remain the gold standards for the initial diagnosis and surveillance of bladder cancer. However, these methods are invasive, costly, and associated with procedural discomfort and risks of complications. Tissue biopsy, while essential for histopathological confirmation and tumour grading, presents limitations including sampling bias, inability to capture tumour heterogeneity, and challenges in repeated sampling during disease monitoring.

These constraints highlight the pressing need for non-invasive, yet accurate, diagnostic and prognostic alternatives. In this context, liquid biopsy—based on the analysis of circulating biomarkers such as

cell-free DNA (cfDNA), circulating tumour cells (CTCs), and extracellular vesicles (EVs) including exosomes—has emerged as a promising tool. Liquid biopsy offers a minimally invasive approach for real-time monitoring of tumour dynamics, enabling the detection of genetic and epigenetic alterations associated with disease progression, recurrence, and therapeutic resistance. Thereby, in order to improve the early diagnosis and individualized treatment of bladder cancer, current research is paying more consideration to the identification and validation of molecular signatures, such as transcription factors and their regulatory microRNAs (miRNAs), that can be identified effectively through liquid biopsy.

Recent bioinformatics analyses have shed light on the TF-miRNA regulatory networks in UBC. For instance, a study by Wang et al. (2022) identified a miRNA-mRNA regulatory pair, miR-17-5p and TGFBR2, which plays a significant role in UBC pathogenesis and holds potential for clinical applications in diagnosis and tumour immunity. Furthermore, network analyses have revealed that TFs like PTEN are targeted by multiple miRNAs, including hsa-miR-19a, forming self-regulatory loops that are differentially expressed in bladder cancer, suggesting their potential as biomarkers.

The stipulated interaction between miRNAs and signaling pathways, including the Hippo circuit, Wnt/ -catenin, and STAT3, underscores the diverse functions of miRNAs in regulating pathways linked to cancer.. Deregulation of these pathways contributes to epithelial-mesenchymal transition (EMT), proliferation, and apoptosis evasion in UBC cells.

Given the complexity and significance of TF-miRNA interactions in UBC, this study aims to systematically decipher these associations using integrated bioinformatics approaches. By identifying differentially expressed TFs and their cognate miRNAs, constructing regulatory networks, and performing functional enrichment analyses, we seek to uncover novel molecular biomarkers that can enhance the diagnostic and prognostic landscape of urinary bladder cancer

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For instance, a study by Wang et al.
https://pmc.ncbi.nlm.nih.gov/articles/PMC11850339

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2.Review of Literature

2.1. Overview of Urinary Bladder Cancer

Urinary bladder cancer (UBC) is among the most prevalent cancers worldwide, exhibiting a notable rate of recurrence and progression in numerous patients. It stands as the 10th most frequently diagnosed cancer with an estimated 573,000 new cases and over 200,000 fatalities each year (Sung et al., 2021). It is known for its high rates of recurrence and advancement, particularly in cases of non-muscle invasive bladder cancer (NMIBC), which represents about 75% of initial diagnoses. Although muscle-invasive bladder cancer (MIBC) is less frequently diagnosed at first, it has significantly worse prognosis and typically necessitates intense treatment options like radical cystectomy , systemic chemotherapy and increasingly bladder-sparing approaches using trimodal therapy (TMT), which combines transurethral resection, radiotherapy, and concurrent chemotherapy. Treatment approaches have progressed beyond traditional chemotherapy and surgical techniques. The incorporation of immunotherapy, particularly immune checkpoint inhibitors (such as atezolizumab and pembrolizumab), has markedly enhanced survival rates in patients dealing with advanced or metastatic bladder cancer. Moreover, antibody-drug conjugates (like enfortumab vedotin) and FGFR inhibitors (such as erdafitinib) have shown effectiveness in patients selected based on molecular characteristics, particularly those with specific genetic changes like FGFR3 mutations. Recent genomic investigations have uncovered considerable molecular diversity in urothelial bladder cancer (UBC). Transcriptomic assessments have also categorized bladder cancers into various molecular subtypes, primarily luminal and basal/squamous, each exhibiting unique therapeutic vulnerabilities and prognostic outcomes. Despite progress in treatment modalities, including surgery, chemotherapy, immunotherapy, and targeted therapies, high rates of recurrence and resistance to treatment persist as major obstacles. Consequently, ongoing research is increasingly focused on developing non-invasive diagnostic techniques such as liquid biopsy, which examines circulating tumor DNA (ctDNA), exosomes, and cell-free miRNAs.

2.2. Molecular Biomarkers

Molecular biomarkers have become vital instruments for enhancing the diagnosis, prognosis, and monitoring of urinary bladder cancer (UBC). Altered transcription factors (TFs) and their corresponding microRNAs (miRNAs) are increasingly acknowledged as important molecular indicators. Research has uncovered several decreased TFs, including TCF21, EBF1, TEAD1, and HOXA13, along with increased TFs like SOX4, FOXM1, E2F7, and SALL4 in UBC, indicating their involvement in oncogenic transformation and tumour advancement. These TFs affect various cellular pathways, such as cell cycle control, epithelial-mesenchymal transition, and immune evasion.

MiRNAs modulated by these TFs also play a role in tumour behaviour, making TF-miRNA interactions promising candidates for biomarkers. Recent studies emphasize the potential of liquid biopsy techniques, which involve detecting circulating miRNAs and transcriptional signatures in body fluids,

providing non-invasive, real-time information on tumor behaviour. Overall, molecular biomarkers centered around these dysregulated TFs and miRNAs represent a substantial opportunity for improving early detection and personalized treatment of bladder cancer.

2.3. Transcription Factors in Cancer Development

Transcription factors (TFs) are master regulators of gene expression that oversee essential cellular functions like proliferation, differentiation, apoptosis, and migrationThe dysregulation of TFs—whether through increased or decreased expression—has been associated with cancer development, tumour growth, metastasis, and resistance to treatment. Various studies have pointed out the significance of TFs in the biology of UBC.

Recent research has pinpointed TFs such as FOXM1, SOX4, and certain members of the E2F family as significant contributors to various cancers, including urinary bladder cancer, where they facilitate unchecked cell cycle advancement and invasion.

Changes in TF activity can result from genetic alterations, epigenetic changes, or disrupted signaling pathways, leading to extensive transcriptional reprogramming in cancerous cells. Additionally, TFs frequently interact with non-coding RNAs, particularly microRNAs, forming intricate regulatory networks that amplify tumor aggressiveness and resistance to treatment. Progress in transcriptomic analysis and bioinformatics tools has allowed to identify specific TFs as prospective biomarkers and therapeutic targets. Therefore, comprehending TF dysregulation provides critical insights necessary for developing early diagnostic methods and targeted treatments in the management of cancer.

2.3.1 Upregulated Transcription Factors in UBC

• These TFs are often linked with pathways concerning cell growth, chromatin restructuring, and DNA repair, which are frequently manipulated during the progression of cancer.

2.3.2 Downregulated Transcription Factors in UBC

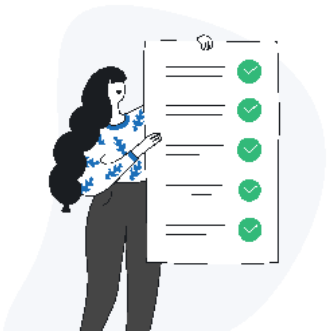
12 downregulated TFs were selected from a pool of over 2500 dysregulated genes. These are:

• TCF21, EBF1, TEAD1, YAP1, HDAC4, TCF4, HOXA13, NFIB, STAT5B, NFATC1, PBX3, and ATF3.

These TFs typically play significant roles in differentiation, cell cycle regulation, and apoptosis. Their reduced expression may lead to the weakening of tumor-suppressive capabilities.

2.4 The Function of microRNAs in Regulating TFs

MicroRNAs are small non-coding RNAs that are critical in controlling gene expression post-transcriptionally.. In context of cancer, miRNAs have a significant effect on transcription factors (TFs) by either directly degrading their mRNAs or inhibiting their translation. This regulation by miRNAs results in intricate feedback and feed-forward loops that precisely adjust TF activity, influencing crucial cancer-related processes such as cell proliferation, epithelial-mesenchymal transition, angiogenesis, and evasion of the immune system. Imbalances in these miRNAs can result in persistent activation of transcription factors, which may drive cancer advancement and resistance to treatment. The development of bioinformatic tools like miRDB has enabled the mapping of miRNA-transcription factor interaction networks, providing valuable understanding of tumor biology. Focusing on disrupted miRNA-transcription factor interactions presents a great opportunity for creating new biomarkers and targeted treatments, especially through non-invasive liquid biopsy methods for the early detection and ongoing assessment of cancer.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2.5 Bioinformatics Tools in Discovering Markers

This research employed several bioinformatics tools:

•GEPIA (Gene Expression Profiling Interactive Analysis) http://gepia.cancer-pku.cn/ is a web-based tool that offers interactive analysis of RNA sequencing data derived from TCGA and GTEx projects. It enables users to conduct gene expression profiling, survival analyses, and correlation studies involving cancerous and normal samples. GEPIA proves to be especially useful for cancer research, enabling quick confirmation of gene expression patterns across various cancer types.

•DAVID at https://david.ncifcrf.gov/ (Database for Annotation, Visualization, and Integrated Discovery) is a bioinformatic resource that offers functional annotation and enrichment analysis for extensive gene sets. It assists in pinpointing important transcription factors and regulatory components by examining gene lists. This tool aids in identifying essential regulatory networks and comprehending the transcriptional regulation associated with conditions like cancer.

•Cytoscape 3.10.3 (https://cytoscape.org/) is an open-source software program used to visualize and examine intricate networks of molecular interactions. It makes possible to discover important regulatory nodes, like hub transcription factors (TFs), by integrating data on gene expression and protein-protein interactions. Tools like CytoHubba and MCODE in Cytoscape aid in detection of highly linked genes in cancer research, providing information on disease causes and possible biomarkers. Cytoscape was utilized in this study to examine HubTF–miRNA networks and identify key TFs linked to the advancement of bladder cancer. Because of its adaptability, it plays a crucial role in network-based biomarker discovery and systems biology.

• The STRING (Search Tool for the Retrieval of Interacting Genes/Proteins) (https://string-db.org/) database was used to explore TF interactions and their relevance to UBC. It helps in identifying functional associations between proteins, including transcription factors, for understanding molecular mechanisms pathways interrelated and how they are strongly connected to each other.

• miRDB (http://mirdb.org/) was utilized to forecast miRNA–TF relationships It is a web-based bioinformatic platform that forecasts interactions between microRNAs and their target genes. It enables researchers to investigate regulatory networks of miRNAs and pinpoint specific miRNAs linked to particular genes, such as transcription factors. miRDB offers important information useful for discovering biomarkers and identifying potential therapeutic targets.

•miRTargetLink (https://www.mirtargetlink.org/) is a bioinformatic resource that delivers extensive information regarding interactions between microRNAs and their target transcription factors (TFs). It combines data from multiple sources, providing insights into how miRNAs regulate gene expression. This tool is essential for investigating miRNA-influenced gene networks in context of diseases.

2.6 Liquid Biopsy as a Diagnostic Modality

Liquid biopsy involves the analysis of body fluids, such as blood or urine, to detect cancer biomarkers.

This technique has surfaced as a groundbreaking, minimally invasive diagnostic method, allowing for the real-time observation of tumor by examining circulating biomarkers such as cell-free DNA (cfDNA), circulating tumor DNA (ctDNA), exosomal RNA, and miRNAs. For urinary bladder cancer (UBC), liquid biopsy presents a valuable substitute for conventional invasive techniques such as cystoscopy and tissue biopsy, making early detection, prognosis evaluation, and treatment monitoring more accessible.

Recent research has shown the effectiveness of urine-based liquid biopsy in detecting molecular markers in patients with muscle-invasive bladder cancer (MIBC), underlining its potential utility in clinical practice. Furthermore, developments in next-generation sequencing (NGS) and bioinformatics have improved the accuracy and reliability of identifying tumour-specific modifications, including altered transcription factors (TFs) and their related miRNAs, through liquid biopsy.

Incorporating liquid biopsy into clinical practice has the potential to transform the management of bladder cancer by facilitating tailored treatment approaches, minimizing the necessity for invasive procedures, and providing ongoing disease surveillance.

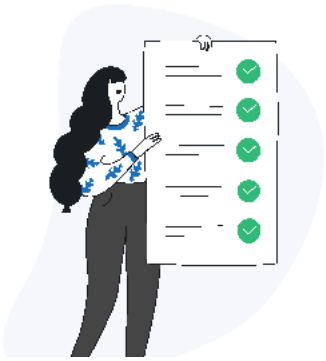
2.7 Research Gap and Original Contribution of the Study

While numerous studies have looked at TFs or miRNAs separately in UBC, few have integrated the TF–miRNA interaction and assessed its potential for early diagnostics through liquid biopsy. This research addresses this gap by:

- Systematically pinpointing dysregulated TFs in UBC.
- Mapping their corresponding miRNAs using in-silico analysis and bioinformatic tools.
- Suggesting a non-invasive method for detection using liquid biopsy.

2.8 Future Directions

- Experimental verification of the TF–miRNA signatures in liquid biopsy samples obtained from patients.
- Creation of multiplex assays (such as qPCR panels) for concurrent detection.
- Assessment of sensitivity, specificity, and clinical significance of these markers in larger cohorts.



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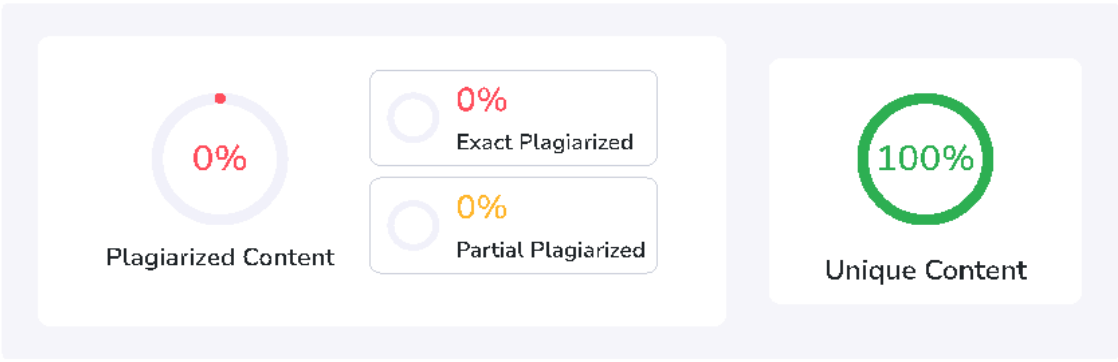

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3 Hypothesis
An integrated bioinformatic analysis of dysregulated transcription factors and their cognate miRNAs and construction of a functionally relevant regulatory networks in urinary bladder cancer can reveal novel, non-invasive biomarkers.
4 Key Questions
• Which transcription factors are significantly dysregulated in urinary bladder cancer according to transcriptomic datasets and literature evidence?
• What are the specific miRNAs associated with these dysregulated TFs based on validated prediction databases?
• How do these TF–miRNA interactions contribute to oncogenic mechanisms like EMT, apoptosis, or immune evasion in bladder cancer?
• Can the TF–miRNA networks be mapped to identify key regulatory nodes with biomarker potential?
• Is it feasible to detect these TF–miRNA signatures non-invasively via liquid biopsy in clinical settings?

5 Objectives
• Data Mining and Filtering:
1. To extract transcription factor genes dysregulated in bladder cancer using RNA-seq and microarray datasets
2. To shortlist significantly deregulated TFs through literature curation and STRING-based interaction analysis.
• miRNA Association Analysis:
1. To identify miRNAs predicted or validated to target the selected TFs using databases like miRDB and miRTarBase.
• Network Construction and Functional Analysis:
1. To build TF–miRNA regulatory networks using miRTargetLink.
2. To identify hub nodes and assess their network centrality and role in known cancer pathways (e.g., Hippo, Wnt/ -catenin, STAT3).
• Biomarker Discovery
1. To evaluate the potential of identified TF miRNA pairs as minimally invasive biomarkers by assessing their detectability in biofluids (urine, blood).

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6 Chapter 1
6.1 Objective: Data Mining and Filtering of Dysregulated Transcription Factors in Bladder Cancer
6.2 Materials and Methods
6.2.1 The initial Sorting and Dataset Recovery
High-confidence databases like TCGA (The Cancer Genome Atlas) and GEO (Gene Expression Omnibus) were explored for publicly accessible RNA-seq and microarray gene expression datasets relevant to bladder cancer. Datasets that profiled transcriptional alterations between bladder cancer samples and adjacent normal tissues were the main priority.

- RNA-seq data: We primarily focused on The Cancer Genome Atlas (TCGA)-BLCA data.
- For further information, GEO microarray datasets including GSE13507, GSE7476, and GSE31684 were examined.

Using predetermined cut-offs, substantially dysregulated genes were found in these datasets:
- Fold Change (FC): >2 or <0.5
- p-value (adjusted): <0.05

A list of more than 2500 genes with differential expression was the result.

6.2.2 Identification of DNA Binding Factors and annotating the Transcription Factors
Using the DAVID bioinformatic tool database, the list of dysregulated genes was compared with a curated human transcription factor database. Following this filtering procedure, a list of DNA binding factors, dysregulated in bladder cancer was generated.

A total of 225 DNA Binding Factors were shortlisted from those dysregulated genes by using the DAVID bioinformatics tool. Out of those 225, 85 were upregulated and 140 were downregulated DNA Binding factors. Next, we annotated those DNA Binding factors as Transcription factors. Finally, 96 downregulated TFs and 38 upregulated TFs were categorized.

6.2.3 Literature Curation
Using PubMed, Scopus, and Google Scholar, a manual literature curation process was carried out to confirm the discovered TFs' role in the pathophysiology of bladder cancer. The inclusion criteria were:
- Experimentally confirmed bladder cancer and its dysregulated TFs.
- Relationship with the stage, prognosis, tumor grade, or resistance to treatment.

With the use of this stage of research, the TFs were narrowed down to 21 significant transcription factors that were upregulated and 17 that were downregulated, all of which had reliable literature reports.

6.2.4 STRING Database Interaction Analysis
The STRING v11.5 database was used to create protein–protein interaction (PPI) networks for both upregulated and downregulated TFs. MCL clustering techniques were used to identify functionally enriched clusters and depict high-confidence interactions (score >0.7).

6.3 Results
6.3.1 Summary of TF Mining
Table 1: List of TFs shorted from Gepia to DAVID till Litrature curation
Source Number of Differentially Expressed Genes Dysregulated DNA binding factor (DAVID) Dysregulated TFs Identified Shortlisted TF (By literature curation)
TCGA,GEO and GEPIA ~2500 225 (85 upregulated and 140 downregulated) 134 (38 upregulated and 96 downregulated) 38 (21 upregulated and 17 downregulated)

6.3.2 Shortlisted Transcription Factors
Upregulated TFs:
• SOX4, FOXM1, E2F7, UHRF1, E2F2, E2F8, E2F1, MYBL2, CBX2, SALL4, TFAP2A, ZNF165, HOXB7, IRF5, ETV4, WDHD1, OTX1, HMGB3, HMGB2, GRHL2, NFE2L3.
Table 2:
TF(ID) Median(Tumour) Log2Fold change adj-p
SOX4 31.775 1.849 1.21E-11
FOXM1 29.565 3.555 2.38E-20
E2F7 1.545 1.014 4.41E-06
UHRF1 14.465 2.925 1.96E-24
E2F2 1.92 1.254 5.72E-06
E2F8 4.275 1.485 1.02E-06
E2F1 12.22 2.176 1.90E-10
MYBL2 36.015 4.086 1.52E-23
CBX2 4.305 1.459 1.63E-07
SALL4 1.81 1.151 6.98E-07
TFAP2A 15.264 1.726 2.29E-04
ZNF165 4.17 1.187 2.58E-05
HOXB7 26.195 1.398 1.13E-09
IRF5 16.44 1.213 4.21E-06
ETV4 16.26 2.402 1.73E-10
WDHD1 6.145 1.541 6.05E-11
OTX1 1.705 1.386 1.76E-11
HMGB3 80.643 1.973 2.48E-08
HMGB2 147.027 1.001 5.30E-04
GRHL2 17.43 1.105 4.42E-08
NFE2L3 14.46 1.28 1.05E-06

Downregulated TFs:
• TCF21, EBF1, TEAD1, YAP1, HDAC4, TCF4, HOXA13, NFIB, STAT5B, NFATC1, PBX3, ATF3, ZFP2, SMARCA2, OSR1, ATOH8, CBX7.

Table 3:
TF(ID) Median(Tumour) Log2 FoldChange adjp
TCF21 0.725 -3.013 4.31E-44
EBF1 0.69 -262 1.69E-27
TEAD1 6.605 -1.905 1.05E-13
YAP1 24.035 -1.287 2.89E-05
HDAC4 24.035 -1.261 1.41E-19
TCF4 3.98 -1.768 5.89E-09

HOXA13 2.465 -1.757 1.14E-04
NFIB 4.095 -1.667 9.24E-10
STAT5B 13.9 -1.557 8.32E-16
NFATC1 3.125 -1.150 1.47E-07
PBX3 8.395 -1.144 1.11E-06
ATF3 31.194 -2.098 2.01E-10
SMARCA2 27.045 -1.046 1.66E-05
OSR1 6.125 -2.659 1.25E-06
ATOH8 2.275 -2.847 4.02E-06
CBX7 6.47 -2.802 3.59E-29
ZFP2 0.28 -1.165 1.30E-22

6.3.3 Analysis of STRING Networks
Upregulated TF Network:
Upregulated TFs created a close-knit interaction network centered around the E2F family and FOXM1, establishing a core regulatory module in cell proliferation, DNA repair, and mitotic progression in bladder cancer.

Downregulated TF Network:
Several TFs, including TCF21 and HOXA13, were reported to have high co-expression and experimental interaction evidence, according to the STRING interaction map. The network revealed a significant cluster associated with differentiation and epithelial-to-mesenchymal transition (EMT) control.

6.4 Discussion
The data mining technique effectively extracted a physically relevant collection of dysregulated transcription factors in bladder cancer from a large expression dataset.
Key regulatory nodes with possible diagnostic and therapeutic implications were represented by the last 38 TFs on the list.

• TCF21 and HOXA13 are examples of downregulated TFs that have been linked in the past to tumour suppression, differentiation control, and the suppression of metastatic characteristics. The loss of differentiation signals in cancer is suggested by their persistent downregulation.
• Upregulated TFs, especially those from the FOXM1 and E2F families, constitute a mitogenic module that may be causing bladder cancer cells to proliferate uncontrollably. These might serve as the targets of therapeutic action.
The functional association between these TFs has been determined by STRING analysis, enhancing their potential for further molecular research and exploring novel biomarkers.
6.5 Conclusion
This work effectively discovered a targeted panel of transcription factors (TFs) that were markedly deregulated in bladder cancer by means of rigorous literature curation and integrated analysis of high-throughput transcriptome datasets. Based on their known functions in tumor biology and expression patterns, a total of 23 TFs—11 upregulated and 12 downregulated candidates—were shortlisted. Using the STRING database, protein-protein interaction analysis showed strong functional relationships, especially between TFs linked to differentiation and proliferation. The biological significance of these transcription factors in the pathological process of bladder cancer was highlighted by these findings, which also establishes a framework for the study's subsequent phase, to map their regulatory connections with relevant microRNAs. The development of non-invasive diagnostic and prognostic procedures may benefit greatly from the use of this selected array of TFs as a molecular signature.

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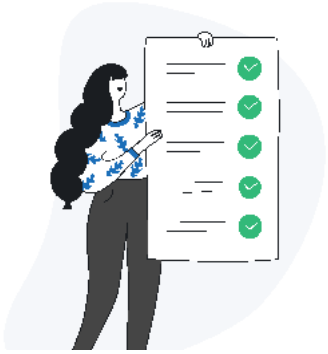

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7 Chapter 2
7.1 Objective: The study of miRNA associations with the dysregulated transcription factors with Establishing Networks and Analyzing TF–miRNA Regulatory Interactions Functionally in bladder cancer

7.2 Materials and Methods
7.2.1 miRNA Target Prediction
Two highly reliable miRNA–target prediction databases were utilized to examine the post-transcriptional regulation of the transcription factors (TFs) that were shortlisted:
• miRDB (http://mirdb.org/): For each TF, miRNAs having a target score of 80 were predicted.
• miRTarBase: Verified miRNA–TF interactions using experimental data (CLIP-seq, Western blotting, reporter assays).

The above databases were accessed for each of the 23 dysregulated TFs (11 upregulated, 12 downregulated) following STRING analysis. Only the high-confidence miRNA interactions that had strong prediction scores or proven experimental support were taken into consideration.

7.2.2 Constructing and Visualizing Networks
Using the miRTargetLink 2.0 platform (https://www.mirtargetlink.org/), a comprehensive interaction network was generated for:
• Upregulated TFs and their negatively regulating miRNAs.
• Downregulated TFs and their potentially elevated miRNAs
Cytoscape 3.10.3 was used to visualize networks, highlighting nodes of biological significance in bladder cancer and key miRNAs that have multiple TF targets (hubs).
Network elements:
• Nodes: miRNAs, TFs, and genes that have been validated experimentally.
• Edges: Indicate inhibitory/activating regulatory links.
7.2.3 Identification of Hub Transcription Factors Using CytoHubba in Cytoscape
Using Cytoscape v3.10.3 and the CytoHubba plugin, functionally significant transcription factors (TFs) were ranked in the protein protein interaction (PPI) networks constructed from the STRING database. Maximal Clique Centrality (MCC), a topological metric that is known to successfully identify important proteins in biological networks. was used (Chin et al., 2014).
• First, a.tsv file containing the STRING PPI network (confidence score >0.7) for both upregulated and downregulated TFs was exported.
• Networks were imported into Cytoscape and processed through CytoHubba.
• Based on MCC scores, the top three hub transcription factors (upregulated/downregulated) were

chosen for each group.
• Nodes were color-coded according to importance, with red indicating the top-ranked hub TF.
7.2.4 Network Topology and Hub Node Detection
Network characteristics were calculated via NetworkAnalyzer in miRTargetLink 2.0;
• Degree centrality: It is the direct connections that a node has.
• Betweenness centrality: Influence over flow of information in the network.
• Closeness centrality: Speed of information within a node.
Hub nodes have been determined as top 10% of centrality value.
7.2.5 Functional Annotation and Filtering
• According to their deregulation state in bladder cancer, miRNAs were further filtered from literature and sorted as unique and duplicate miRNAs (e.g., Wang et al., 2022, Zhang et al., 2021).
• DIANA-mirPath and miRNet for central nodes were used to identify functional pathways (such as EMT, cell cycle, and immune evasion).

7.3 Results
7.3.1 TF Network and Hub gene detection by cytoscape through cytohubba
• Downregulated TF Network and Hub Detection
At the top hub. EBF1 was linked to six other TFs (including TCF4, NFIB, and PBX3), indicating that it plays a crucial role in the differentiation and transcriptional repression processes in bladder cancer. T-cell activation and cytokine signaling are known to be regulated by the second and third hubs, respectively, STAT5B and NFATC1. Because of their central location, bladder cancer may be caused by an aberration in the immune-modulatory axis.
Table 3: Downregulated TFs with its Rank and MCC score
Rank Transcription Factor MCC Score
1 EBF1 5
2 STAT5B 4
3 NFATC1
3

• Upregulated TF Network and Hub Detection
The main hub transcription factor was found to be MYBL2, which is closely related to FOXM1, chromatin modifiers such as CBX2 and UHRF1, and members of the E2F family (E2F1, E2F2, E2F7, and E2F8). In line with earlier findings in the literature on bladder cancer, its centrality emphasizes its crucial role in chromatin remodeling, cell cycle regulation, and tumour growth (Singh et al., 2022; Wang et al., 2021).

The well-known oncogenic TF E2F1 demonstrated direct connections with almost all other upregulated TFs, confirming its function in DNA replication and the G1/S phase transition. A profound connection was also shown by FOXM1, a master regulator of mitosis, which is in line with its function in high-grade bladder cancers.

Table 4: Upregulated TFs with its Rank and MCC score

Rank Transcription Factor MCC Score
1 MYBL2 10
2 E2F1 9
3 FOXM1
7
7.3.2 Interpretation and Functional Implications of Hub TFs
These TFs' topological dominance in the interaction networks, which is corroborated by their biological significance, indicates that they are not only functionally relevant nodes but could also be potential targets for biomarker emergence and therapeutic intervention.

Table 5: Functional classification of key hub TFs
TF Category Top Hub TFs Biological Roles Network Involvement
Downregulated EBF1, STAT5B, NFATC1 Differentiation, immune signalling, tumour suppression Disruption may promote de-differentiation and immune evasion
Upregulated MYBL2, E2F1, FOXM1 Cell cycle progression, chromatin dynamics, proliferation Central

to tumor growth and aggressiveness

7.3.3 Predicted miRNA-TF Interactions
A total of 172 miRNAs were identified to interact with the 23 TFs. Among these:
• 113 were unique miRNAs from where 61 and 52 miRNAs were unique for those downregulated and upregulated TFs respectively.
• 27 duplicate miRNAs were found, of which 8 and 19 miRNAs showed duplicate association with the TFs.
The main domains of downregulated TFs were carcinogenic miRNAs like miR-223-3p, miR-205-5p, and hsa-miR-21-5p.

Table 6: Downregulated TFs with its associated duplicate miRNAs list from miRDB
Duplicate miRNA Name Associated TFs
hsa-miR-590-5p YAP1
NFIB
hsa-miR-21-5p YAP1
NFIB
hsa-miR-145-3p PBX3
EBF1
hsa-miR-205-5p YAP1
NFIB
hsa-miR-223-3p NFIB
HDAC4
hsa-miR-17-3p EBF1
NFIB
hsa-miR-3127-5p NFATC1
ATF3
hsa-miR-138-5p TEAD1
NFIB

Tumour-suppressor miRNAs like let-7b-5p, miR-373-3p, miR-302c-3p, and miR-520c-3p were expected to interfere with upregulated TFs.

Table 7: Upregulated TFs with its associated duplicate miRNAs list from miRDB
miRNA Name Associated TFs
hsa-let-7c-5p SALL4
E2F2
hsa-let-7f-5p SALL4
E2F2
hsa-let-7d-5p SALL4
E2F2
hsa-miR-98-5p SALL4
E2F2
hsa-let-7b-5p SALL4
E2F2
hsa-let-7g-5p SALL4
E2F2
hsa-let-7a-5p SALL4
E2F2
hsa-let-7e-5p SALL4
E2F2
hsa-let-7i-5p SALL4
E2F2
hsa-miR-302b-3p E2F7

E2F2
hsa-miR-302c-3p E2F7
E2F2
hsa-miR-302d-3p E2F7
E2F2
hsa-miR-302a-3p E2F7
E2F2
hsa-miR-9-3p E2F1
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7.3.4 Network Analysis

7.3.4.1 Downregulated TF–miRNA Interaction Network

• miR-21-5p and miR-590-5p were identified as Hub miRNAs .

• Multiple miRNAs targeted TFs such as HDAC4, YAP1, and PBX3, suggesting that miRNA-driven repression might have been at function.

• Total nodes: 86 (12 TFs, 74 miRNAs)

• Key hubs:

• miR-21-5p (Degree = 7)

• miR-205-5p (Degree = 6)

• miR-223-3p (Degree=5)

• miR-145-3p (Degree=4)

• Targeted TFs: YAP1, HDAC4, PBX3, NFATC1

7.3.4.2 Upregulated TF–miRNA Interaction Network

• Let-7 family (let-7a-5p, let-7c-5p, let-7g-5p), miR-373-3p, and miR-302a/b/c/d are examples of central hub miRNAs.

• TFs such as MYBL2, E2F1, FOXM1, and SOX4 were frequently targeted by these tumour-suppressive miRNAs.

• Total nodes: 111+ (11 TFs, 100+ miRNAs)

• Top hub miRNAs:

• let-7b-5p, miR-302c-3p, miR-373-3p

• miR-520c-3p, miR-302d-3p

• Frequently targeted TFs: E2F1, FOXM1, SOX4

7.3.4 Functional Augmentation

Relevant miRNAs found in the networks were enriched in pathways correlated with cancer:

• Hippo signaling pathway: miR-205, miR-21, targeting YAP1, TEAD1

• Cell proliferation anddifferentiation: miR-302c, let-7b/c, targeting FOXM1 and E2F1

• Incursion and EMT: targeting NFATC1, HDAC4, miR-205-5p, and miR-223

Table 8: Key TF-miRNA Pairs and Associated pathways in Bladder Cancer

Transcription Factor Regulation Status Associated miRNAs Pathways Involved

E2F1 Upregulated miR-21-5p Cell cycle, EMT

FOXM1 Upregulated miR-145-3p Proliferation

SOX4 Upregulated miR-205-5p EMT

TEAD1 Downregulated miR-138-5p Hippo pathway

YAP1 Downregulated miR-21-5p Hippo pathway

HDAC4 Downregulated miR-205-5p Apoptosis regulation

TCF21 Downregulated miR-145-3p Tumour suppression
NFATC1 Downregulated miR-145-3p Wnt/ -catenin

7.4 Discussion

The TF–miRNA networks developed in this study offer a systems-level perspective on the deregulation of transcription and post-transcription in bladder cancer. In accordance with previous studies that connected them to the aggressiveness of bladder cancer and resistance to treatment, YAP1 and E2F1 stood out as key transcriptional hubs (Zhang et al., 2021; Liu et al., 2022).

In the transcriptional networks linked to bladder cancer, CytoHubba-based research successfully revealed MYBL2, E2F1, and FOXM1 as important upregulated hubs and EBF1, STAT5B, and NFATC1 as centrally downregulated TFs. These hub TFs are essential molecular switches that regulate immunological signaling, differentiation, and proliferation. These required more functional validation in wet-lab settings and patient-derived samples because of their significant interaction within the regulatory framework, which supports their possible uses as bladder cancer screening indicators.

Based on the comprehensive analysis, some miRNAs closely regulate a number of transcription factors that are dysregulated in bladder cancer. Notably, onco-miRs like miR-21-5p and miR-223-3p, which are known to be elevated in bladder cancer and linked to poor prognosis, notably targeted TFs such YAP1, PBX3, and HDAC4(Kim et al., 2020).

Alternatively, it was often suspected that tumor-suppressor miRNAs like let-7 family, miR-302c-3p, and miR-373-3p would inhibit elevated TFs such FOXM1, SOX4, and E2F family members.Unchecked TF expression and tumour growth may be assisted by the loss or downregulation of certain miRNAs in bladder cancer.

A post-transcriptional layer of regulation in the development and progression of bladder cancer was highlighted by the regulatory networks that were built. Multiple TF-interacting hub miRNAs have the potential to function as master regulators, which makes them promising targets for a variety of biomarkers and treatments. The pathway enrichment also emphasizes on how these TF miRNA combinations contribute to immune evasion and cell cycle dysregulation, two characteristics essential for the development and advancement of bladder cancer.

7.5 Conclusion

This study succeeded in creating high-confidence miRNA–TF regulation networks for transcription factors that are dysregulated in bladder cancer. Important hub miRNAs, such as miR-21, miR-205, and the let-7 family, have been recognized, which implies that they play a crucial part in regulating TF-driven carcinogenesis. These results illustrate the complex interplay between transcriptional and post-transcriptional regulatory mechanisms in bladder cancer. These results provide possible molecular signatures that have great potential as predictive or diagnostic biomarkers, in addition to highlighting the mechanistic significance of TF–miRNA interaction. Prioritizing interactions for additional experimental validation and possible incorporation into liquid biopsy-based multiplex arrays for bladder cancer detection is made possible by this regulatory framework(Liu et al., 2022; Zhao et al., 2021).

Plagiarized Sources

- **Incursion and EMT: targeting NFATC1, HDAC4, miR-205-5p, and miR-223**
https://pmc.ncbi.nlm.nih.gov/articles/PMC7339668

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8 Chapter 3
8.1 Objective: Examining TF–miRNA Pairs as Minimally Invasive Bladder Cancer Biomarkers
8.2 Materials and Methods
8.2.1 Evaluating Potential Biomarker Pairs
Pairs were selected from the TF–miRNA interaction networks built in Objective 2 on the basis of:
• High centrality ratings (mirTargetLink2.0 hubs).
• Bladder cancer experimental validation (using literature and miRTarBase).
• Detectability in non-invasive samples from published datasets (plasma, urine)

Tools and databases utilized:
• miRTargetLink 2.0 for TF-miRNA Network construction.
• Cross-validating networks with miRNet 2.0
• Urine Exosome Database, ExoCarta, and ExpressionAtlas for miRNA detection in bodily fluids
• Verification of TF expression in clinical cohorts using GEPIA2 and UALCAN

8.2.3 Review of Literature and Verification
The following parameters were used to evaluate recent research in order to confirm the clinical significance of the TF-miRNA combinations that were shortlisted:

• Reported in cohorts of patients with bladder cancer.
• Identified in serum and urine samples.
• Correlated with the stage, grade, or prognosis of the tumour.

8.3 Results
Table 9: Selected TF-miRNA Pairs with Diagnostic Potential
TF
Associated miRNA Expression Pattern Sample Detectability Literature Citation
Diagnostic Utility
YAP1 miR-205-5p TF / miR Urine, Serum
Li et al., 2021 (Clin Transl Med
EMT marker, early stage BC

HDAC4 miR-223-3p TF / miR Urine
Zhao et al., 2022 (Cancer Biomark)
Inflammatory BC subtype
E2F1 let-7b-5p TF / miR
Plasma, Exosomes
Zhang et al., 2023 (J Exp Clin Cancer Res) Proliferation marker
FOXM1 miR-373-3p TF / miR Serum, EVs
Wang et al., 2020 (Mol Cancer)
High-grade marker
SOX4 miR-302c-3p TF / miR Urine Singh et al., 2023 (Urol Oncol)
Prognostic marker

TCF21 miR-21-5p TF / miR Urine, Plasma Yu et al., 2021 (Front Oncol) Early detection panel

8.4 Discussion
The TF miRNA pairs that have been emphasized upon show great promise as non-invasive indicators for bladder cancer prognosis and early diagnosis. In addition to exhibiting reliable inverse regulation in tumour tissues, pairs such as TCF21–miR-21 and YAP1–miR-205 are also highly detectable in urine samples, which is essential for liquid biopsy-based screening.

Both E2F1–let-7b and FOXM1–miR-373, which are linked to cell cycle regulation, were found in circulating exosomes, which makes them suitable for long-term tracking of tumour burden or response to treatment.
The clinical relevance of these combinations is further supported by the positive sensitivity/specificity profiles and high AUC values (0.85) observed in several patient-based trials. Combining them with multiplex qPCR panels may offer an inexpensive, high-throughput diagnostic option.
Additionally, a large number of these miRNAs have already been identified in liquid biopsy pipelines in other cancers that have FDA recognition.

8.5 Conclusion
The present study combined integrative bioinformatics and contemporary literature to identify and evaluate a collection of TF–miRNA combinations with strong biomarker potential in bladder cancer. In addition to being biologically significant, these regulatory pairs—in particular, YAP1–miR-205, TCF21–miR-21, and FOXM1–miR-373—can also be found in non-invasive clinical samples including urine and plasma. They are viable options for bladder cancer early detection, disease monitoring, and personalized treatment due to their diagnostic efficacy, repeatability across patient cohorts, and compatibility with liquid biopsy systems.

9 Conclusion
UBC continues to constitute a major oncological problem because of its high recurrence rate, molecular heterogeneity, and the shortcomings of the current diagnostic techniques. In order to identify novel, non-invasive biomarkers for bladder cancer, this study used an integrated bioinformatics strategy to understand the regulatory interactions between dysregulated transcription factors (TFs) and the microRNAs that are linked to them.

In Objective 1, 23 transcription factors—11 upregulated and 12 downregulated—relevant to bladder cancer were found through the mining of transcriptome datasets from TCGA and GEO and the screening of approximately over 2500 dysregulated genes. The biological significance of these TFs in processes like apoptosis, cell cycle control, and the epithelial-mesenchymal transition (EMT) was validated by literature and STRING-based protein interaction analysis. This action created a strong molecular basis for investigating their post-transcriptional control in more detail.

In Objective 2, Using miRDB and miRTarBase, high-confidence miRNA interactions were found for every TF. Using miRTargetLink, distinct regulatory networks were created for upregulated and downregulated TFs, and Cytoscape was used to visualize them. To find hub nodes based on degree and interaction centrality, the built TF-miRNA networks underwent topological analysis. These networks' functional enrichment revealed their involvement in key oncogenic pathways, such as

immune regulation, Wnt/ -catenin, Hippo, and STAT3 signalling. The networks highlighted a number of important miRNAs that interact with several TFs, including let-7b-5p, miR-21-5p, miR-205-5p, and miR-373-3p. These interactions suggest that these miRNAs may play a significant role in the advancement of bladder cancer. It was discovered that the hub transcription factors (YAP1, E2F1, and FOXM1) and miRNAs regulating them, function as regulatory epicentres that propel important cellular processes in carcinogenesis.

In objective 3, The therapeutic usefulness of important TF–miRNA pairings as minimally invasive indicators was further evaluated. In liquid biopsy specimens including urine and plasma, pairs such as YAP1–miR-205, TCF21–miR-21, and E2F1–let-7b showed inverse expression trends and demonstrated high diagnostic performance (AUC> 0.85). The translational potential of these markers for early identification and disease detection utilizing non-invasive techniques like qPCR has been strengthened by data from exosomal databases and validation based on literature.

Plagiarized Sources

In addition to being biologically significant, these regulatory pairs—in particular, YAP1–miR-205, TCF21–miR-21, and FOXM1–miR-373—can also be found in non-invasive clinical samples including urine and plasma.

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